# HETEROCYCLES AS MEDIATORS IN THE TRANSFORMATION OF ORGANIC STRUCTURES

BY

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1989

To my husband and daughter

ACKNOWLEDGMENTS

I am ever grateful to my research director, Professor A. R. Katritzky, for his guidance, kindness, patience, and support throughout this research work, and for empathetic understanding, without which I could not have brought up my daughter and completed this program of study.

I express my profound gratitude and appreciation to Dr. W. M. Jones for the contributions he made to the accomplishment of this study. I am indebted to my committee members, Dr. W. M. Jones, Dr. M. Battiste, Dr. K. Wagener, Dr. A. Toth and Dr. N. Bodor for their invaluable suggestions, assistance and cooperation.

I wish to thank my teachers, Dr. W. M. Jones, Dr. R. W. King, Dr. M. Battiste, Dr. Y. Ohrn, Dr. R. J. Hanrahan, Ms. C. Drum and Dr. G. Schmid. Without them providing me a good start I would not have completed this study program.

I am extremely grateful to Drs. J. Lam, P. Savage, D. Yannakopoulou and S. Perumal for their earnest help, many useful comments, and constructive criticism to improve the contents of this dissertation, and I am very appreciative of the time they have spent towards this.

I wish to thank Drs. C. Marson, S. Sengupta, G. Rewcastle, B. Krawietzka and L. Urogdi for their contributions towards my research work. It is a pleasure to thank all of my coworkers through these years of study.

My sincere gratitude is also extended to the faculty and staff of the chemistry department for their understanding and cordial relationship.

At this time I wish to acknowledge the Oberlin Shansi Program for selecting me as an exchange faculty, which enabled me to pursue my graduate studies in the United States. I greatly acknowledge Lady Doak College (where I studied and worked) for providing the environment to inspire the quest for academic excellence.

I have very special place in my heart for Miss Shanti Manuel, Dr. Linde Katritzky and Mrs. Elizabeth Jones for being with me and supporting me in crisis. I also thank my friends here in the States and in India.

I thank God for giving me an adorable mother, admirable father, affectionate brother and sisters and their families, who never gave up hope in my academic ventures. I thank Viji and Mr. Rajaram for their encouragement and full involvement in sending me abroad to further my studies.

Last, but not least, I am deeply grateful to my husband for the understanding, support, persuasion given to me, and for typing the manuscript. A multitude of gratitude is extended to my daughter who stood behind me graciously and patiently during the long period of my graduate studies.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Abstract of a Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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AUGUST, 1989

Chairman: Alan R. Katritzky
Major Department: Chemistry

The efficiency of two heterocyclic compounds, pyrylium salt and benzotriazole, as mediators in the transformations of organic structures was investigated.

Pyrylium salts are known to transform primary amines into their corresponding pyridinium salts. Applying this principle in the synthesis of N-(5-amino-5-carboxyl-n-pentyl)-pyridinium salts from lysine was successful. This lead to the structural elucidation of Glycophorin A, and confirmation of the involvement of lysine residues in cotransporter protein.

The nucleophilicity of the benzotriazole anion was used to prepare hydroxyethyl derivatives of benzotriazole from 2-chloroethanol. Hydroxyethylbenzotriazole was further transformed into haloethylbenzotriazoles. Subsequent displacement of chloride ion by sulfur and nitrogen nucleophiles allowed access to a range of synthetically useful synthons.

Functionalization of N-monosubstituted formamides into alkyl isocyanides has been successfully executed with benzotriazole as the α-activating group. The synthesis of N,N,N'-trialkylformamidines, via isocyanides possessing benzotriazole as an α-leaving group, made the method versatile. Application of the same method for the synthesis of N,N'-disubstituted formamidines was unsuccessful.

# CHAPTER 1

## GENERAL INTRODUCTION

Transformation of the carbon skeleton and functional group(s) of an organic molecule is a challenging and exciting area of organic chemistry. Such transformations can be facilitated by mediators or mediating groups. The requirement of a mediator or mediating group overlap with that of protective groups: (i) they should be easily synthesized from commercially available or readily prepared starting materials, (ii) introduction of these groups must be facile, (iii) they should remain unaffected under various synthetic transformations of the molecule in which they are introduced, and (iv) they should be easily removed.

The most contrasting feature between mediating groups and protecting groups is that, unlike the protective group, mediating groups are introduced at a necessary position where they can facilitate the transformation of the molecule. Protective groups are always introduced on the functional groups. Another difference is that protecting groups are always removed after the transformation sequence

is accomplished. But the mediating groups need not be removed if the mediating groups in the transformed molecule have some applications in industry, agriculture or medicine.

In laboratory syntheses, heterocyclic compounds are frequently a source of latent functionality. The ring systems can be carried through many stages of a synthetic sequence, and then can be cleaved (if necessary) to produce the transformed molecule. Aromatic heterocyclic compounds are generally more stable than aliphatic heterocyclic compounds. The two heterocyclic compounds chosen as mediating groups are pyrylium salts and benzotriazole, and both are aromatic heterocycles.

Although pyrylium salts are used as versatile synthons in organic synthesis, their use in protein modification is very limited. They are used as specific blocking reagents of the ε-amino group in the side chain of α-aminoacids. The pyridinium formed is stable under the conditions of biological molecules. Although it blocks the ε-amino group of lysine, the reacted pyrylium is not removed. It is used, not only to transform the side chain, but also to ascertain the overall structure of the protein.

Benzotriazole is commercially available and inexpensive. Its easy introduction into the carbon skeleton, stability, electron withdrawing nature, and α-activation has been extensively investigated by Katritzky

and his coworkers. In addition, benzotriazole can be easily cleaved and displaced by a variety of methods. Using all these properties of benzotriazole, a versatile transformation in the skeleton by forming C-C, C-H, C-N, C-Br, C-Cl, and C-S bonds, and in the functional groups by forming amino, isocyano, and amidine groups has been developed.

Although only two aromatic heterocycle mediators were investigated, their application in the transformation involves a variety of organic structures. Hence, an elaborate introduction of the relevant area is given in each of the corresponding chapters. Introductory material in all forthcoming chapters embraces the concept of mediating groups with an emphasis on mediating group criteria and transformations to be achieved.

# CHAPTER 2

# MODIFICATION OF PROTEINS USING PYRYLIUM SALTS

## 2.1. Introduction

Proteins are complex organic molecules to which a unique linear aminoacid sequence (the primary structure) imparts a unique pattern by the folding of the polypeptide chain (the secondary and tertiary structures) in three dimensions. Elucidation of the structure of proteins is concerned with analysis of the linear arrangement of aminoacids and of the spatial arrangement of the polypeptide chain. Chemical modification is a major tool in both approaches. Although such intentional modification is widely performed in biochemical studies, it is also advantageously applied in the fields of food technology and pharmacology, for the development of new and improved products.

### 2.1.1. The Chemistry of the Chemical Modification of Proteins

Generally the chemical reactivity of proteins arises from the reactivity of the side-chains of the aminoacids. These side-chains can function as sulfur, nitrogen, and

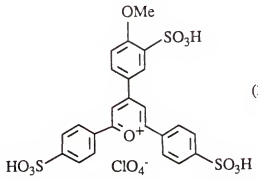
oxygen nucleophiles in both addition and displacement reactions. They may also be involved in oxidation and reduction reactions. Nucleophilic reactions of aminoacid side-chains are usually strongly pH dependent and are often investigated using acylating or alkylating reagents.

2.1.1.1. Modification of amino groups in proteins

Lysine is one of the major constituents of proteins and is usually located on the "surface" of the molecule, in contact with solvent and readily accessible to chemical reagents. In addition to the ε-amino group of lysine, the only other primary amine function occurring in proteins is the α-amino group of the N-terminal residue. The ε-amino group is a strong base of pKa = 10.2 [74MI151], whereas the α-amino group is less basic, as shown by its pKa = 7.8 [74MI151]. Because of these ionization characteristics, specific ε-amino group modifications are carried out at higher pH. Many of these have been acylations giving amide derivatives with properties depending on the reagent that is used. Recently, alkylations have become prominent; these include reactions with haloacetals, aryl halides, and aromatic sulfonic acids. On the other hand, reductive alkylations have been achieved by exposing the protein to an aldehyde or ketone in the presence of a reducing agent such as sodium borohydride, sodium cyanoborohydride [79MI451], or amine boranes [81MI345]. Most of these reagents are not very specific to an ε-amino group.

2.1.1.2. Pyrylium salts as ε-amino group modifiers

Pyrylium salts have been employed as specific reagents for modifying the ε-amino group of lysine. The ε-amino group of lysine in chymotrypsin and acetoacetate-decarboxylase [71JAM3530] has been modified by 2,4,6-trimethylpyrylium perchlorate (2.1a) to form the corresponding N-substituted pyridinium salts with only a few of the total number of available lysine residues.




(2.2)

Chymotrypsin and gelatin, with 30 and 14 lysine residues respectively, has been modified by 4-(4-methoxy-3-sulfophenyl)-2,6-bis-(4-sulfophenyl)pyrylium perchlorate (2.2) [84JCS(P2)875]. In each of these cases the reaction kinetics were studied using ultraviolet spectroscopy (u.v.), under pseudo first order conditions, in which the effective lysine concentration was at least 30 times that of the pyrylium salt.

Application of the same method [71JAM3530, 84JCS(P2)875] to high molecular weight proteins, with lesser numbers of lysine residues, or to membrane intact proteins,

may be less successful. Proteins which have absorption maxima overlapping with the administered pyrylium, or the formed pyridinium, may also be less easy to modify.

### 2.1.2. Glycophorin

#### 2.1.2.1. Structure of Glycophorin

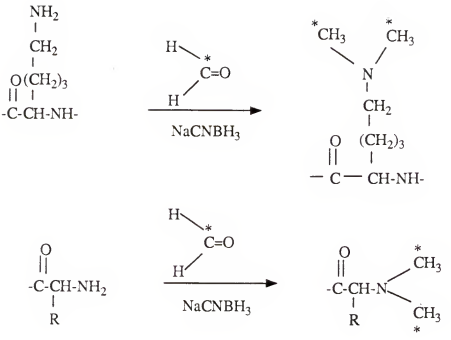
Human erythrocyte membrane has three types of glycophorins namely glycophorin A,B and C. Glycophorin A is preponderant among the three and is responsible for the expression of MN blood-group determinant. It is a transmembrane protein, with a molecular weight of 31,000, traversing from the exterior to the interior of the cell. It has a protein moiety and a carbohydrate moiety. The former has 131 aminoacid residues with three domains: (i) aminoacid residues 1-70 form the N-terminal region which lies in the external surface of the membrane, (ii) residues 71-92 are the hydrophobic portion of glycophorin which appears to be embedded in the phospholipid of the membrane, and (iii) residues 93-131 protrude into the cytoplasm of the cell [81MI221].

Glycophorin A exists in two types and denoted glycophorin $A^M$ and $A^N$. Glycophorins $A^M$ and $A^N$ differs in the aminoacid residues at positions 1 and 5. Glycophorin $A^M$ contains serine and glycine and $A^N$ has leucine and

glutamic acid at positions 1 and 5, respectively. Both glycophorin $A^M$ and $A^N$ have five lysine residues, of which two, at positions 18 and 30, lie in the N-terminal region, and three residues, at positions 100, 101 and 108, lie in the C-terminal region of the glycophorin. Fifteen carbohydrates residues are found as O-linked tetrasaccharides, which are composed of D-galactose, D-GalNAc and D-NeuAc and, in one complex, a N-linked oligosaccharide.

### 2.1.2.2. Anomalous dimerization of glycophorin $A^M$ in solution

Although the discovery of the MN blood-group was made by Landsteiner and Levine in 1927, large scale investigations on the mode of expression of MN blood-group determinants on the red blood cell surface began only in the last decade [75MI183, 80JBC2744]. This brought out the controversy concerning the structures of the M and N determinants displayed by glycophorin $A^M$ and $A^N$, which focused on three functional groups: the lysine residues, the N-terminal aminoacid residues and the alpha-D-NeuAc residues. To gain insight into the structural differences that exist between glycophorin $A^M$ and $A^N$, Dill's group at Clemson University has done extensive studies on native and isolated glycophorin by reductive $^{13}C$ methylation (Scheme 2.1) of $\alpha$- and $\varepsilon$-amino groups.




Scheme 2.1

Reductive $^{13}C$ methylation (Figure 2.1) on glycophorin $A^M$ and $A^N$ showed a singlet at 44.1 ppm for all ten methyl carbons of N,N-di($^{13}C$)methylated ε-amino group of lysine residues. Same result was observed both in solution and in native glycophorin $A^M$ and $A^N$. In partial reductive $^{13}C$ methylation study, $^{13}C$ resonance for α-N,N-dimethylleucine was observed at 42.8 ppm in glycophorin $A^N$ intact and in solution (Figure 2.2 C and D). On the other hand $^{13}C$ resonance for α-N,N-dimethylserine was observed at 43.3 ppm only in intact glycophorin $A^M$ (Figure 2.2 A). For glycophorin $A^M$ in solution, two resonances were observed: the resonance at 43.3 ppm was attributed to α-N,N-

dimethylserine and the resonance at 42.8 ppm was the resonance in question for assignment (Figure 2.2 B). Such an anomaly was postulated as dimerization between two glycophorin molecules via a head to head coupling [85BBA396]. Although the results exclude the occurrence of dimerization via the ε-amino groups of the lysine residues near the C-terminus, they do not exclude the possibility of intermolecular crosslinking via lysine near the N-terminus of one molecule and N-terminal serine of another molecule. At this juncture, the use of a selectively blocking ε-amino group reagent (for example a pyrylium salt) would confirm whether there is ser-lys cross link or ser-ser cross link between two N-termini of glycophorin $A^M$.

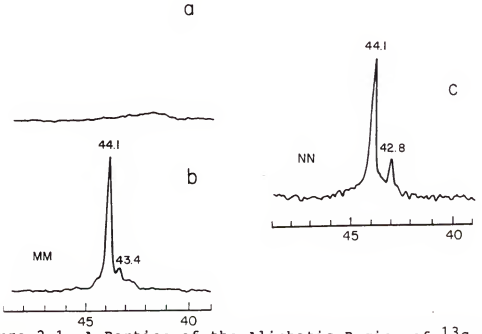



Figure 2.1 A Portion of the Aliphatic Region of $^{13}C$ n.m.r. of Nonmethylated (a) and Fully ($^{13}C$)-Methylated Intact Glycophorin A [(b) and (c)].

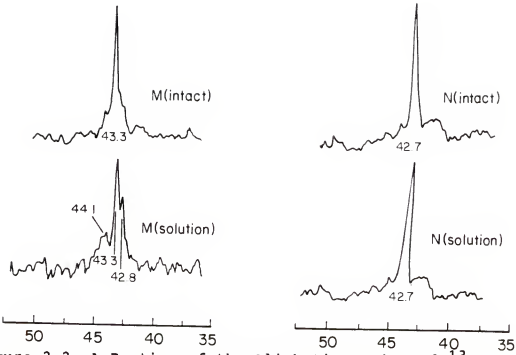



Figure 2.2 A Portion of the Aliphatic Region of $^{13}C$ n.m.r. of Partially Reductively $^{13}C$-Methylated Glycophorin A in Solution.

2.1.3. Cotransporter Protein

2.1.3.1. Structure and mode of action of cotransporter protein

The active transportation of glucose into the intestinal and renal epithelial cells is performed by a cotransporter, or symtransporter protein in the brush border membrane. The molecular weight of the cotransporter is 74,000 [84JBC14105]. Recently the primary and secondary structure of the cotransporter protein has been determined using expression cloning and cDNA sequencing [87N379]. Likely candidates for the participating lysyl residues in glucose transport of the cotransporter exist on its major

hydrophilic domain extending from the outer membrane. This domain possesses a cluster of lysyl residues at positions 134, 139, 157, 235, 254, 342, 412, 415, 416, 420 and 478. Steven's group (Physiology department at the University of Florida) is actively involved in investigating the detailed mechanism of action of the cotransporter [82MI213, 84MI417, 88MI1021].

The mode of transport of glucose is coupled with sodium ion transport. Sodium ion binds to the carrier protein, thereby increasing the carrier's affinity for glucose. Glucose at the surface of the cell then binds to the carrier site and the ternary complex (sodium ion, carrier and glucose) undergoes a conformational change across the cell membrane. When the ternary complex meets the cytoplasm where potassium ion concentration is high, the sodium ion on the complex is displaced by potassium ion. This lowers the affinity for glucose, hence glucose is discharged into the cytoplasm.

### 2.1.3.2. Role of lysine residues in glucose transport

Information concerning the molecular events involved in sodium ion alteration of carrier glucose affinity is incomplete, although it is known that the cotransporter protein is inactivated by various reagents including phenyl

isothiocyanate, 5,5'-dithiobis-(2-nitrobenzoic acid), 4,4'-(diisothiocyano)stilbene-2,2'-disulfonic acid, and also by reductive methylation [83MI429, 83MI437, 84MI2223, 87MI5790, 88MI343, 88MI1021]. Based on these inhibitors it is thought that a putative critical lysine residue is required for glucose transport or phlorizin (substrate analogue) binding activity. However, these reagents fail to definitively assign a functional role to lysine due to their partial reactivity to α-amines, thiols, and other functional groups in the side chains of aminoacids. Use of a sterically bulky, and water soluble, pyrylium salt would selectively block the ε-amino group of lysine and would confirm the role of lysine in sodium dependent glucose transport.

## 2.2. Objectives

The ultimate goal of this investigation was to circumvent the limitations in the existing method in using pyrylium salts for modifying the protein as discussed in section 2.1.1.2. This could be achieved by Chemical and Biochemical approaches.

To summarize, the objectives are (i) to synthesize N-(5-amino-5-carboxyl-n-pentyl)-2,4,6-trimethylpyridinium (2.3a-c) salts with various gegen ions on a preparative scale from pyrylium salts (2.1) and lysine, which would also

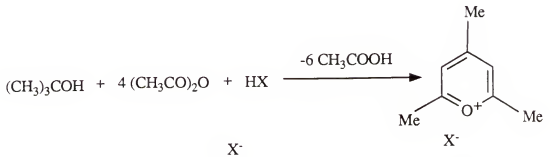
provide a direct evidence for pyridinium salts (2.3a-c) formation as well as it would narrow down the problem of stoichiometry of reaction with protein; (ii) to synthesize various pyrylium salts which cater to the needs of biochemical studies after the preliminary results with (2.1a) and proteins; (iii) to modify glycophorin $A^M$ using pyrylium salts (2.1) with appropriate gegen ions in order to differentiate the two possible dimeric states of glycophorin $A^M$ in solution and to increase the efficacy of the ε-amino group blocking in glycophorin by increasing steric hindrance at the nucleophilic centres C-2 and C-6 of (2.1) (based on these results, as well as from the available literature, the best useful pyrylium salt for modifying glycophorin needs to be selected as well as the conditions need to be standardized); and (iv) to modify the sodium dependent glucose cotransporter protein in brush border membrane of kidney and intestine by pyrylium salts. In this work the pyrylium salt is used to probe the critical functional lysine residues of the transporter protein.

## 2.3. Results and Discussion

### 2.3.1. Synthesis of Pyrylium Salts

Pyrylium salts (2.1a-c) were prepared from tertiary butanol, acetic anhydride and the conjugate acid of the

desired gegen ion using literature methods [73OSC(V)1106, 1112 and 1114], as shown in Scheme 2.2.




(2.1)

a $ClO_4$

b $BF_4$

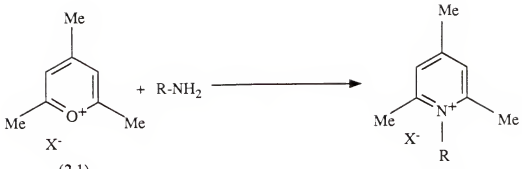
c $CF_3SO_3$

Scheme 2.2

The structure of the pyrylium salts were characterized by $^1H$ n.m.r. and $^{13}C$ n.m.r. spectroscopy.

## 2.3.2. Synthesis of Pyridinium Salts

Using more polar solvents (for example, acetic acid) in the preparation of pyridinium salts (2.3 b and c) and model compounds (2.3 d-g) exhibited differences in yield between the tetrafluoroborate and the trifluoromethane sulfonate products (Scheme 2.3). This is due to the isolation problem caused by the more hygroscopic nature of (2.3 c, e and g).




(2.1) (2.3)

| | $X^-$ | R |
|---|---|---|
| a | $ClO_4$ | $HOOC(NH_2)CH\text{-}(CH_2)_4$- |
| b | $BF_4$ | $HOOC(NH_2)CH\text{-}(CH_2)_4$- |
| c | $CF_3SO_3$ | $HOOC(NH_2)CH\text{-}(CH_2)_4$- |
| d | $BF_4$ | $CH_3CH_2CH_2CH_2$- |
| e | $CF_3SO_3$ | $CH_3CH_2CH_2CH_2$- |
| f | $BF_4$ | $C_6H_5$ |
| g | $CF_3SO_3$ | $C_6H_5$ |

Scheme 2.3

The N-(5-amino-5-carboxyl-n-pentyl)-2,4,6-trimethyl-pyridinium salts (2.3 a-c) were successfully prepared in high yield from lysine and the corresponding pyrylium salt in ethanol (Scheme 2.3). The melting point of the known compound (2.3a) agreed with the literature value. The structures were characterized also by $^1H$ and $^{13}C$ n.m.r. spectroscopy. All three pyridinium salts (2.3 a-c) are very hygroscopic.

These results give direct evidence that reaction between (2.1) and lysine occurs and hence (2.1) could be administered to modify proteins. The pyrylium salt (2.1 c) is better for proteins than (2.1 a) since (2.1c) is more soluble in water than (2.1a). Poor solubility is one of the reasons for incomplete modification of the protein.

### 2.3.3. Modification of Glycophorin $A^M$

#### 2.3.3.1. Reaction of (2.1c) with model compound, tuftsin acetate

Tuftsin acetate was chosen as a good model system to determine the molar ratio of blocking reagent (2.1c) to protein in order to selectively block the ε-amino group of lysine completely and not the α-amino group of the N-terminus residue, threonine. It was determined that 2.5 :1.0 mole ratio of pyrylium salt (2.1c) to tuftsin acetate provides high degree of selectivity for ε-amino group.

From this result it was calculated that a molar ratio of 12.5:1.0, the pyrylium salt (2.1c) to glycophorin $A^N$ (as control) would be required in order to fully block the ε-amino groups of lysine residues.

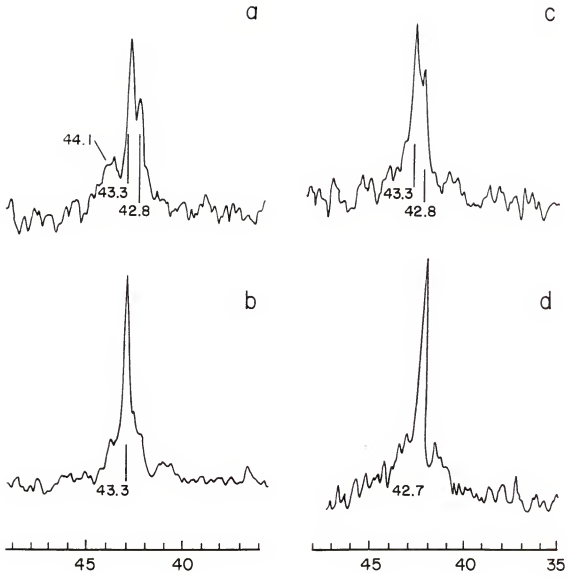



Figure 2.3 A Portion of the Aliphatic Region of $^{13}C$ n.m.r. of Partially Reductively $^{13}C$-Methylated Glycophorin; a = $A^M$ in Solution, b = $A^M$ in Intact Erythrocyte, c = $A^M$ Pretreated with Pyrylium in Solution and d = $A^N$ Pretreated with Pyrylium in Solution.

2.3.3.2. Reaction of (2.1c) with glycophorin $A^M$ and $A^N$

It was determined that a 15:1 molar ratio was necessary to modify all the lysine residues in glycophorin $A^N$. The requirement of a larger molar ratio for the protein than the model system could be attributed to steric reasons which exist in large molecules, like proteins. The extent of modification was monitored by aminoacid analysis.

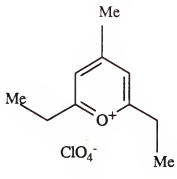
Reductive $^{13}C$ methylation was then performed on the pyrylium salt-treated glycophorin $A^M$ and $A^N$ in solution. The results of $^{13}C$ n.m.r. are shown in Figure 2.3. It is remarkable that in both spectra in Figure 2.3 (c) and (d), the resonance previously seen at 44.15 ppm due to N,N-di($^{13}C$)methyllysine is absent. This indicates that (2.1c) has successfully blocked the ε-amino groups of all five lysine residues of glycophorin $A^M$ and $A^N$ and thus prevented them from being $^{13}C$ labelled during the reductive methylation reaction. In Figure 2.3, (d) has only one resonance at 42.7 ppm which is attributed to the N,N-di($^{13}C$)methylleucine residue which agreed with the previous $^{13}C$ labelling studies [85BBA396] done in both intact erythrocytes and solution of glycophorin $A^N$. In Figure 2.3, (c) has two resonances at 43.3 ppm and 42.8 ppm as has been previously observed for $^{13}C$ labelled studies of glycophorin $A^M$ in solution (Figure 2.2). These resonances can be assigned to the methyl groups attached to the N-terminal

serine residue of glycophorin $A^M$ in solution not pretreated with pyrylium. These results not only exclude the possibility of the dimeric state due to the N-terminal serine and the lysine residue at position 15 or 30, but definitely prove that the dimeric state is due to the cross linking between N-terminal serine residues as head to head of two glycophorin $A^M$ that occurs during the reductive $^{13}C$ methylation of Glycophorin $A^M$. The fact that such cross linking is seen only in gloycophorin $A^M$ and not in $A^N$ solution indicates that the three-dimensional structure about the N-terminus of glycophorin $A^M$ is indeed unique, and is different from the structure about the N-terminus of glycophorin $A^N$.

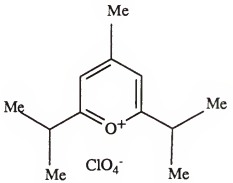
2.3.3.3. Effect of steric factor

When a ten-fold excess of (2.1c) was used, the N-terminal serine α-amine was also blocked. The decrease in selectivity at higher concentration of pyrylium (2.1c) could possibly be overcome by increasing steric hindrance at the two nucleophilic centers C-2 and C-6 of (2.1c). The α-amino group of the N-terminus is closer to the back bone of the protein, hence it is in a relatively more crowded environment than the ε-amino group of lysine, which is in the side chain and away from the back bone. Therefore, the

increase in steric hindrance at the nucleophilic centers of pyrylium (2.1c) might selectively block lysine residues of $A^M$ and $A^N$. If this were so, it would indicate the structural difference at the N-terminus region of both. Hence 2,6-bisethyl-4-methylpyrylium perchlorate (2.4) and 2,6-bisisopropyl-4-methylpyrylium perchlorate (2.5) were prepared using a previously reported method [59A(625)74] and were used to modify lysine residues selectively in glycophorin $A^M$ and $A^N$.




(2.4)




(2.5)

2.3.3.4. Selection of (2.2) as the most suitable pyrylium salt

The increase in steric bulkness at positions 2 and 6 as well as an increase in solubility, selectively blocked the ε-amino group rather than α-amino group of the N-terminus aminoacid. This selectivity has been proved by using 4-(4-methoxy-3-sulfophenyl)-2,6-bis(4-sulfophenyl)pyrylium perchlorate (2.2) (Figure 2.4). The optimum conditions to selectively block the ε-amino groups of lysine residues in

glycophorin, with pyrylium salts, was determined to be a molar ratio of 30:1.

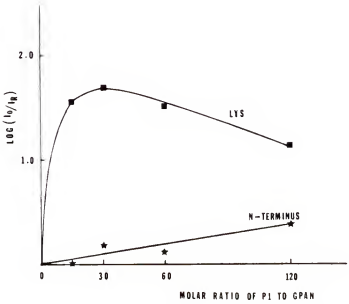



Figure 2.4 Estimated Ratio of Pyrylium Salt (2.2) to Glycophorin.

2.3.4. Modification of Na/Glucose Cotransporter by 4-(4-Methoxy-3-sulfophenyl)-2,6-bis(4-sulfophenyl)pyrylium perchlorate (2.2)

2.3.4.1. Pyrylium inhibition of the cotransporter

The sodium-dependant transport of glucose into membrane vesicles was inhibited with increasing concentrations of pyrylium (2.2), as shown by the data in Figure 2.5. Exposure to 2.5 mM pyrylium for 30 min. inhibited uptake activity by 90%, compared to control exposure (carbonate

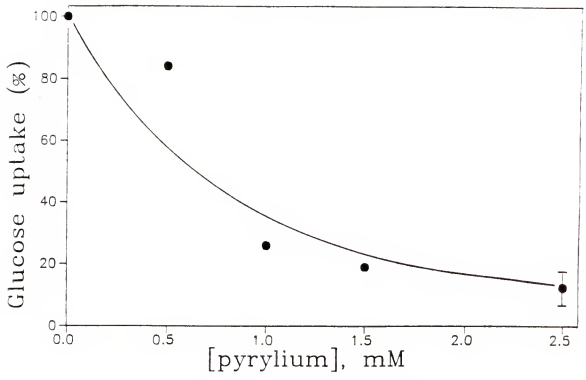



Figure 2.5 Pyrylium Inhibition of $Na^+$-Dependent Glucose Transport.

buffer only, no pyrylium). The data in Figure 2.5 demonstrate that the pyrylium salt inhibits sodium-dependent phlorizin (substrate analogue) binding activity also, and thus confirms that lysine residues are required for activity.

2.3.4.2. Cotransporter conformational influence on pyrylium inhibition

Within Figure 2.6, the shaded databars further indicate that when the transport substrates, sodium ion plus glucose were bound to the cotransporter during the reaction with low dosage (0.5 mM) pyrylium, there was an additional pyrylium inhibition of phlorizin binding activity. It is known that

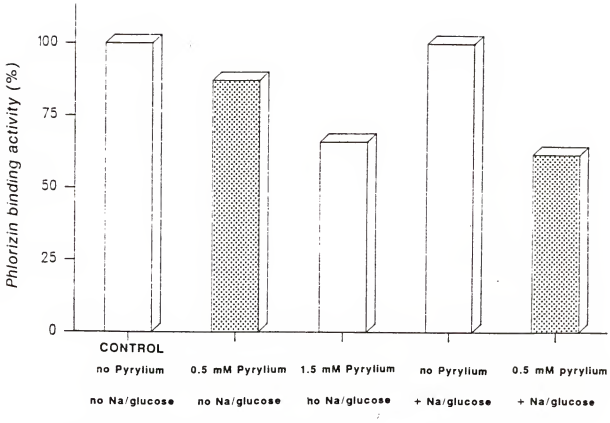



Figure 2.6 Pyrylium Inhibition of Cotransporter Phlorizin Binding Activity.

the bound substrates induce a specific conformational shift [87MI5790, 88MI1021]. Therefore, the cotransporter substrate-enhanced pyrylium inhibition binding (Figure 2.6) confirms the premise that a critical lysine residue(s) on the cotransporter is exposed during this conformational shift.

## 2.4. Conclusions

Synthesis of N-(5-amino-5-carboxyl-n-pentyl)-2,4,6-trimethyl pyridinium (2.3a-c) salts with various gegenions gave the direct evidence of product formation.

The use of pyrylium salts as ε-amino blocking reagent proved unequivocally that there is a head to head cross linking of the two glycophorin $A^M$ molecules by way of the two N-terminal L-serine residues. Reactions with increased steric bulkiness at the C-2 and C-6 positions of the pyrylium salt (2.1) further proves that there is difference in the N-terminus region of glycophorin $A^M$ and $A^N$. Reactions with (2.2) show that it can be considered as a specific blocking reagent for lysyl residues in protein modification studies.

The use of (2.2) confirms the involvement of lysine residue(s) in sodium ion-dependent cotransporter activity in the brush border membrane of the intestine and the kidney.

## 2.5. Experimental

Melting points were determined on a Bristoline hot-stage microscope and are uncorrected. $^1H$ n.m.r. spectra were recorded on a Varian EM 360L spectrometer with sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as an internal

reference. $^{13}C$ n.m.r. spectra were recorded on a Jeol FX-100 (25 MHz) spectrometer. Elemental analyses were performed under the supervision of Dr. R. W. King of the University of Florida Department of Chemistry.

### 2.5.1. Synthesis of Pyrylium Salts

#### 2.5.1.1. General procedure for 2,4,6-trimethylpyrylium salts

Tertiary butanol (2 mol) acetic anhydride (10 mol) and the conjugate acid of the gegenion (1.75 mol) gave (2.1) [73OSC(V)1106, 1112 and 1114].

2,4,6-Trimethylpyrylium perchlorate (2.1a), yield 66%, yellow prisms (acetic acid:diethylether = 1:1) m.p. 246-249°C; lit. m.p. 244°C [73OSC(V)1106]. $^1H$ n.m.r. δ ($D_2O$) 2.7 (s, 3H), 3.0 (s, 6H), 8.0 (s, 2H).

2,4,6-Trimethylpyrylium tetrafluoroborate (2.1b), yield 46%, pale yellow granules (ethanol:methanol = 1:1) m.p. 224-227°C; lit. m.p. 224-226°C [73OSC(V)1112]. $^1H$ n.m.r. δ ($D_2O$) 2.7 (s, 3H), 3.0 (s, 6H), 7.9 (s, 2H).

2,4,6-Trimethylpyrylium trifluoromethane sulfonate (2.1c), yield 42%, pale yellow granules (dioxane: acetic acid = 7:1) m.p. 119°C; lit. m.p. 119-120°C [73OSC(V)1114]. $^1H$ n.m.r. δ ($D_2O$) 2.8 (s, 3H), 2.9 (s, 6H) and 8.0 (s, 2H). $^{13}C$ n.m.r. δ ($D_2O$) 23.1, 25.3, 125.6, 177.2, 180.4.

2.5.1.2. General procedure for 2,6-bisalkyl-4-methylpyrylium perchlorate (2.4) and (2.5)

Tertiary butyl chloride (0.1 mol), aluminum chloride (0.1 mol), the corresponding acid chloride (0.2 mol) and perchloric acid (0.12 mol) gave (2.4) and (2.5) [59A(625)74].

2,6-Diethyl-4-methylpyrylium perchlorate (2.4), yield 45%, yellow crystals (water) m.p. 189-191°C; lit. m.p. 189°C [59A(625)74]. $^{1}H$ n.m.r. δ ($D_2O$/TFA) 1.1 (t, 6H), 2.5 (s, 3H), 2.9 (q, J=7 Hz, 4H), 7.5 (s, 2H). $^{13}C$ n.m.r. δ ($D_2O$/TFA) 9.6, 27.5, 121.6, 174.6, 181.5 (methyl carbon at C-4 not seen).

2,6-Di-i-propyl-4-methylpyrylium perchlorate (2.5), yield 48%, pale green crystals (water) m.p. 173°C, lit. m.p. 173°C [59A(625)74]. $^{1}H$ n.m.r. δ ($D_2O$/TFA) 1.5 (d, J=7 Hz, 12H), 2.6 (s, 3H), 3.0-3.6 (m, 2H), 7.6 (s, 2H). $^{13}C$ n.m.r. δ ($D_2O$/TFA) 19.6, 23.2, 33.5, 120.9, 174.5, 183.2.

2.5.1.3. Synthesis of 4-(4-methoxy-3-sulfophenyl)-2,6-bis(4-sulfophenyl)pyrylium perchlorate (2.2)

3-Formyl-6-methoxybenzene sulfonic acid (5 mmol), 4-acetylbenzene sulfonic acid (10 mmol) and perchloric acid (4g of 70% solution) gave 65% (2.6), m.p. >350°C; lit. m.p. >350°C [84JCS(P1)857].

2.5.2. Synthesis of Pyridinium Salts

2.5.2.1. General procedure for the synthesis of (2.3a-c)

A solution of lysine monohydrochloride (5 mmol) in sodium hydroxide (2 mL of 1N), or free lysine (5 mmol) was added to ethanol (10 mL). To this solution the pyrylium salt (2.3) (5 mmol) was added while the reaction mixture was stirred. After heating at 40°C for 1.5 h the solvent was removed under reduced pressure. The solids that were obtained were very hygroscopic.

N-(5-Amino-5-carboxyl-n-pentyl)-2,4,6-trimethyl-pyridinium perchlorate (2.3a): Lysine monohydrochloride and (2.1a) gave (77%) yellow glassy flakes, m.p. 144-147°C, lit. m.p. 147°C [71JAM3530]. $^{1}H$ n.m.r. δ ($D_2O$) 1.5-2.3 (m, 6H), 2.7 (s, 3H), 2.8 (s, 6H), 3.0-3.6 (m, 2H), 3.8-4.7 (m, 1H), 7.7 (s, 2H).

N-(5-Amino-5-carboxyl-n-pentyl)-2,4,6-trimethyl-pyridinium tetrafluoroborate (2.3b): Lysine monohydrochloride and (2.1b) gave (74%) white powder, m.p. 169-170°C. $^{1}H$ n.m.r. δ ($D_2O$) 1.0-2.7 (m, 6H), 2.7 (s, 3H), 2.9 (s, 6H), 2.7-3.3 (m, 2H), 3.7-4.0 (m, 1H), 7.9 (s, 2H). $^{13}C$ n.m.r. δ ($D_2O$) 23.2, 24.1, 29.1, 32.3, 41.8, 57.1, 125.7, 131.1, 177.0, 180.5.

N-(5-Amino-5-carboxyl-n-pentyl)-2,4,6-trimethyl-pyridinium trifluoromethane sulfonate (2.3c): Lysine monohydrochloride and (2.1c) gave (76%) yellow brown flakes, m.p. 167-180°C. $^{1}H$ n.m.r. δ ($D_2O$) 1.0-2.4 (m, 6H), 2.6 (s, 3H), 2.8 (s, 6H), 3.0-3.3 (m, 2H), 3.7-4.0 (m, 1H), 7.7 (s, 2H).

2.5.2.2. General procedure for the synthesis of (2.3d-g)

To a solution of the primary amine (2.2) (10 mmol) in glacial acetic acid (10 mL), was added the corresponding pyrylium salt (2.1b or 2.1c) (10 mmol) in small portions. After refluxing for 40 min, the reaction mixture was filtered. Addition of diethyl ether (5 mL) resulted in an oil which was then dissolved in cold ethanol (5 mL) and filtered. Dilution of the filtrate with diethyl ether afforded the solid.

N-(n-Butyl)-2,4,6-trimethylpyridinium tetrafluoroborate (2.3d): n-Butylamine and (2.1b) gave (74%) white needles, m.p. 77-78°C. For $C_{12}H_{20}BF_4N$ required C, 54.33; H, 7.50; N, 5.20; Found C, 54.13; H, 7.74; N, 4.93. $^{1}H$ n.m.r. δ ($D_2O$) 0.8-2.5 (m, 5H), 2.5 (s, 3H), 2.9 (s, 6H), 3.0-3.3 (m, 2H), 4.6 (t, J=6Hz, 2H), 7.8 (s, 2H). $^{13}C$ n.m.r. δ ($D_2O$) 15.4, 22.0, 22.7, 23.2, 32.4, 130.9, 156.6, 160.5.

N-(n-Butyl)-2,4,6-trimethylpyridinium trifluoromethane sulfonate (2.3e): n-Butylamine and (2.1c) gave (22%) white powder, m.p. 66-70°C. For $C_{13}H_{20}F_3NO_3S$ required C, 47.70; H, 6.16; N, 4.28; Found C, 47.46; H, 6.14; N, 3.87. $^1H$ n.m.r δ ($D_2O$) 0.3-2.0 (m, 7H), 2.5 (s, 3H), 2.9 (s, 6H), 4.5 (t, J=7 Hz, 2H), 7.7 (s, 2H). $^{13}C$ n.m.r. δ ($D_2O$) 15.4, 22.0, 22.7, 23.2, 32.4, 54.5, 130.9, 156.5, 160.5.

N-(Phenyl)-2,4,6-trimethylpyridinium tetrafluoroborate (2.3f): Aniline and (2.1b) gave (76%) white flakes, m.p. 112-114°C; lit. m.p. 90-91°C [81CCC584]. For $C_{14}H_{16}BF_4N$, required C, 59.13; H, 5.63; N, 4.93; Found C, 59.22, H, 5.64; N, 4.68. $^1H$ n.m.r δ ($D_2O$) 2.3 (s, 6H), 2.7 (s, 3H), 7.7 (s, 2H), 7.9 (s, 5H). $^{13}C$ n.m.r. δ ($D_2O$) 23.7, 23.9, 129.8, 129.9, 133.6, 133.8, 141.1, 157.6, 162.6.

N-(Phenyl)-2,4,6-trimethylpyridinium trifluoromethane sulfonate (2.3g): Aniline and (2.1c) gave (27%) white needles, m.p. 73-75°C. For $C_{15}H_{16}O_3F_3NO_3S$ required C, 51.90; H, 4.65; N, 4.04; Found C, 51.62; H, 4.64; N, 3.86. $^1H$ n.m.r. δ ($D_2O$) 2.3 (s, 6H), 2.7 (s, 3H), 7.7 (s, 2H), 7.9 (s, 5H). $^{13}C$ n.m.r. δ ($D_2O$) 23.7, 23.9, 129.9, 128.1, 133.1, 133.6 141.1, 157.5 and 162.7.

### 2.5.3. Materials for Glycophorin Modification

Modification of glycophorin using pyrylium salts was investigated at Clemson University by Dill's group.

$^{13}C$ enriched formaldehyde (97.8% $^{13}C$ enriched) was purchased from Merck and Company Inc. as a 20% aqueous solution. Sodium cyanoborohydride was obtained from Sigma Chemical Company Inc. and rabbit anti-M and anti-N sera were obtained from Ortho Diagnostic Systems, Inc. Outdated human blood was obtained from Carolina Regional Blood.

### 2.5.4. Materials for Cotransporter Modification

Modification of cotransporter using pyrylium salts was investigated by Steven's group in the Physiology Department of University of Florida.

Brush border membrane vesicles were prepared from rabbit jejunal mucosal enterocytes, utilizing a standard $Ca^{2+}$ aggregation technique [84MI417, 82MI213]. The membrane vesicles were suspended in 300 mM mannitol and 5 mM HEPES/Tris (pH 7.5). Membranes were enriched about 15-20 fold in the marker enzymes, alkaline phosphatase and leucine aminopeptidase, compared to the total cell homogenate [82MI213].

All reagents were obtained from Sigma Chemical Co. (St. Louis, MO), or Aldrich Chemical Co. (Milwaukee, WI), and radioisotopes were obtained from New England Nuclear (Wilmington, DE). All measurements represent the means of at least three replicates.

2.5.5. Methods for Glycophorin Modification

Homozygous (MM and NN) red blood cells were typed by hemagglutination assays using rabbit anti-M and anti-N sera. Isolation of Glycophorin A using literature procedures [75MI113, 78MI87] gave yields of 35-50 mg of glycophorin for each pint of blood used. This represents approximately 70 to 80% of the total glycophorin found in the red cell membrane.

Fully reductively $^{13}C$ methylated glycophorin A was obtained as follows. The protein (50 mg) was dissolved in distilled deionized water (5 mL) and to that 50 fold excess of $^{13}C$ enriched formaldehyde was added. The pH was adjusted to 7.5. Sodium cyanoborohydride (20 mmol) was then added and the pH was readjusted whenever necessary. The reaction mixture was stirred at 4°C for 12 h and was then exhaustively dialyzed against distilled water. The protein was then freeze-dried.

Partial $^{13}C$ methylation of glycophorin $A^M$ and $A^N$ was performed in a similar manner as outlined above. However, here a limited amount of $^{13}C$ enriched formaldehyde was added (2:1 mole ratio $^{13}C$ enriched formaldehyde : glycophorin A) and the pH of the reaction was kept at pH 7.0 in order to effect preferential methylation of the N-terminal aminoacid.

The pyrylium salt was dissolved in 0.01 N hydrochloric acid solution and transferred into the carbonate buffer solution (pH 10.0) containing glycophorin A (20 mg). The reaction mixture was left at room temperature for 48 h and then the solution was dialyzed against distilled water with an Amicon DIAFLO ultrafiltration cell equipped with a PM-10 membrane. A 20% aqueous solution of $^{13}C$ enriched formaldehyde (50$\mu$L) was added to the pyrylium modified glycophorin solution and after 20 min sodium cyanoborohydride (30 mg) was added. The reaction mixture was left at 10°C for 8 h. The reaction solution was then dialyzed against distilled water.

$^{13}C$ NMR spectra were recorded on JEOL-FX90Q at 22.49 MHz in the Fourier transform mode. The NMR samples were prepared by the addition of deionized distilled water (1.0 mL) to the lyophylisate and the pH was adjusted to 7.0, monitored with a Radiometer PHM 63 pH meter. The sample was contained in 10mm NMR tube with a 5mm tube containing (90:10) acetone-deuterium oxide solution to serve as the field frequency lock. The spectra were taken at 25°C for all samples. For all $^{13}C$ NMR spectra the relative intensities of the resonances of the lysyl residues and the N-terminal amino groups were measured relative to the resonance of the external acetone which was used as an integration standard. Chemical shifts are reported relative to 1,4-dioxane (66.5 ppm).

2.5.6. Methods for Cotransporter Modification

A freshly prepared stock solution of pyrylium (in 0.01 N HCl) was added to N-α-acetyl-L-lysine methyl ester, or 2 mg of membranes in buffer containing (final reaction): potassium carbonate (pH 10.1) (100 mmol), EDTA (1 mmol), and pyrylium salt (ranging from 0 to 2.5 mmol). Membrane reaction times were fixed at 30 min, at 22°C. Appropriate controls (i.e., buffer without pyrylium) were run in parallel in each case. Reactions were occasionally monitored with a Beckman DU7-HS spectrophotometer. Membrane reactions were stopped by adding 30 mL of ice cold mannitol (300 mmol), 25 mM HEPES/Tris (pH 7.5), whereupon membranes were centrifuged at 40,000 x g for 30 minutes. The washed pellets were resuspended in buffer containing 300 mM mannitol and 25 mM HEPES/Tris (pH 7.5). Protein concentrations were determined using the BioRad method (BioRad, Richmond, CA).

Glucose transport and phlorizin binding activities of the $Na^+$/glucose cotransporter in membrane vesicles were assessed at 22°C by standard techniques [84MI417, 82BBA557]. Transport was measured by 8 sec uptakes of 50 $\mu$M [$^3$H]D-glucose measured in the presence of extravesicular sodium chloride (100 mmol), or in choline chloride (100 mmol). Phlorizin binding activity was assessed by measuring the 60

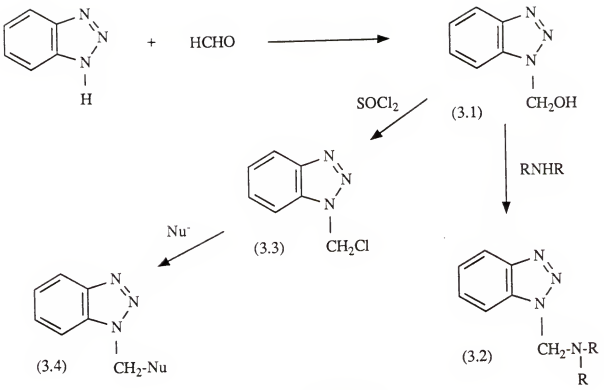
sec binding of 0.25 $\mu$M [$^{3}$H]phlorizin in the presence of sodium chloride or choline chloride (100 mmol). Sodium-dependent uptake or binding was equal to the total uptake or binding measured in sodium chloride minus uptake or binding in choline chloride.

CHAPTER 3

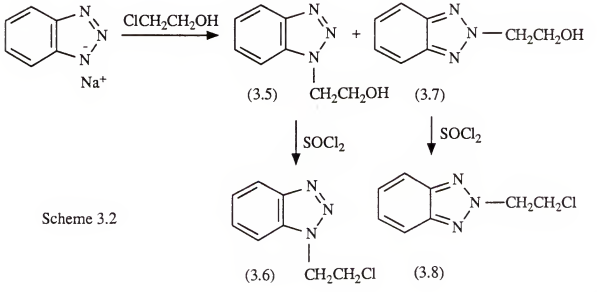
SYNTHESIS OF β-HETEROATOM LINKED ETHYLBENZOTRIAZOLES

## 3.1. Introduction

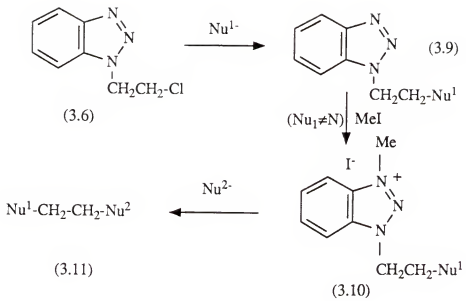
Investigation of the synthesis and application of α-heteroatom substituted methylbenzotriazole has been extensively and systematically studied recently by Katritzky et. al. [87JCS(P1)781, 791, 799, 805, 811]. Hydroxymethylbenzotriazole (3.1) which is an intermediate and is obtained from benzotriazole and formaldehyde, plays an important role in further transformations (Scheme 3.1). Hydroxymethylbenzotriazole (3.1) reacts readily with dialkylamines or diarylamines to give aminomethylbenzotriazole derivatives (3.2). Such aminomethylbenzotriazoles possess various biological activities [68MI1814, 78MI63, 80MI291] and they are also used as additives for insulating and lubricating oils [81USP4278553, 80USSRP761543, 81JP81163195, 81USP4264436]. The reaction of (3.1) with thionyl chloride affords chloromethylbenzotriazole (3.3) which possesses two acidic protons, which could eventually be substituted by two electrophiles, and two potential leaving groups which could be substituted by two nucleophiles [87JCS(P1)781]. Substitution by nucleophiles such as oxygen, sulfur, and




Scheme 3.1




Scheme 3.2

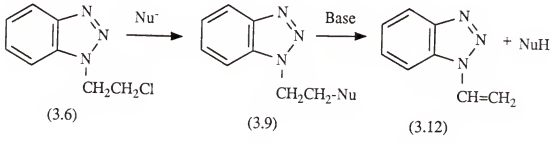



Scheme 3.3

phosphorus [87JCS(P1)781, 87JCS(P1)811] affords α-heteroatom substituted benzotriazoles (3.4) which may be further modified by electrophilic substitutions.

A few β-heteroatom substituted benzotriazoles are known [57MI1, 38CB596, 65MI248] and these were mainly synthesized for pharmacological purposes. A systematic investigation of β-heteroatom substituted benzotriazole derivatives could open a new approach in organic synthesis. It is known that the sodium salt of benzotriazole reacts with 2-chloroethanol to give both the 1- and the 2-isomers of β-hydroxyethylbenzotriazole (3.5) and (3.7) [38CB596], which further react with thionyl chloride to give β-

chloroethylbenzotriazoles (3.6) and (3.8), respectively (Scheme 3.2) [57MI1]. This is a good analogy to the reaction of hydroxymethylbenzotriazole (3.1) and hence potential nucleophilic displacement of chloride ion in (3.6) to give (3.9) is possible. Furthermore, the displacement of benzotriazole may be achieved via the quaternary salt (3.10)




Scheme 3.4

to give (3.11) (Scheme 3.3). Since the α-protons adjacent to the benzotriazole group are acidic, electrophilic substitution may be feasible after lithiation.

Another important aspect of ethyl derivatives of benzotriazole is that they may be used as protecting groups by exploiting the properties of α-activation of benzotriazole and the heteroatom at the β-position (Scheme 3.4). Heteroatom containing nucleophiles may be protected as (3.9) by displacing the chlorine atom from (3.6).

Further treatment of (3.9) with a suitable base would abstract the α-proton which would lead to the elimination of nucleophile as well as formation of vinylbenzotriazole (3.12). Hence benzotriazole could be used as a protecting group and at the final step can be removed as vinyl benzotriazole (3.12). Similar studies were done by Katritzky et. al. on 4- and 2-vinylpyridines [84TL1223, 86JOC4914].

## 3.2. Objectives

The goal of this study was to synthesize β-haloethylbenzotriazole via hydroxyethylbenzotriazole, and then to perform nucleophilic displacements on the haloethyl derivative to obtain other β-heteroatom linked ethylbenzotriazole derivatives.

## 3.3. Results and Discussion

### 3.3.1. Synthesis of β-Hydroxyethylbenzotriazoles (3.5) and (3.7) and β-Haloethylbenzotriazoles (3.6), (3.8), (3.13) and (3.14)

Both isomers, 1-(β-hydroxyethyl)benzotriazole (3.5) and 2-(β-hydroxyethyl)benzotriazole (3.7) are obtained in yields of 57% and 20%, respectively, from the sodium salt of

benzotriazole and 2-chloroethanol. Figure 3.1 shows the numbering system in 1- and 2-substituted benzotriazole. The greater formation of the 1-isomer over the 2-isomer is due to the nucleophilicity of the N-1 (or N-3) compared to the N-2 atom in the benzotriazole anion, which is reflected in their electron density and charge density [75JCS(P2)1695, 78JHC127] at these N-atoms (Figure 3.1).


Figure 3.1 Numbering in 1- and 2-Substituted Benzotriazoles.

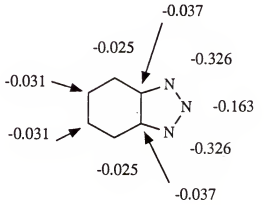
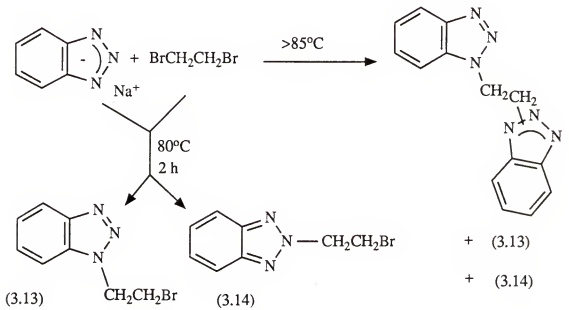



Figure 3.2 Electron Density in Benzotriazole Anion (double bonds not shown).

Once the 1- and 2-isomers are formed, they do not isomerize in solution unlike the case of aminomethylbenzotriazole derivatives. The mechanism of this isomerization has been discussed previously [75JCS(P1)1181]. The 1- and 2-isomers were separated and each was treated with thionyl chloride in chloroform to give the 1- and 2-isomers of chloroethylbenzotriazoles (3.6) and (3.8) in 81% and 87% yield, respectively. Although the 1-isomer of chloroethylbenzotriazole (3.6) was known, the corresponding 2-isomer (3.8) was not, therefore it was fully characterized by $^{13}C$ and $^{1}H$ n.m.r. spectra and elemental analysis.




Scheme 3.5

The bromoethylbenzotriazoles (3.13) and (3.14) were prepared from one equivalent of sodium salt of benzotriazole and with two equivalents of ethylene bromide in an overall

yield of 58% (Scheme 3.5). Separation of the 1- and 2-isomers was difficult. In addition, prolonged heating at 85°C or heating at temperatures higher than 85°C, or the use of equivalent amount of reactants resulted in 1,2-(bisbenzotriazolyl)ethanes along with (3.13) and (3.14). Reported methods for the synthesis of (3.13) and (3.14) involve two steps, an initial synthesis of the hydroxy derivatives (3.5) and (3.7) and further reaction of the individual isomers (3.5) and (3.7) with an eight fold excess of HBr with heating for 40 hours [38CB596]. Because of the difficulties encountered in optimizing the conditions as well as isolating the isomers, for further transformations as revealed in Scheme 3.3, chloroethylbenzotriazole (3.6) was used. Consequently (3.8) can also be used for similar transformations.

For (3.5) and (3.7) the melting points agreed with the reported literature melting points [38CB596]. $^{13}C$ and $^{1}H$ N.m.r. data also add more evidence to the structure and these are not available in the literature. The carbon chemical shifts of the benzotriazole ring (Tables 3.1 and 3.3) were assigned by reference to those previously reported for 1-substituted and 2-substituted benzotriazoles [83H1787]. The characteristic $^{13}C$ pattern of 1-substituted derivatives for (3.5), (3.6) and (3.13) and 2-substituted benzotriazole derivatives for (3.7), (3.8) and (3.14) were observed.

Table 3.1 $^{13}C$ N.m.r. Chemical Shifts (δ) of β-Hydroxyethyl- (3.5) and (3.7), and β-Haloethyl- (3.6), (3.8), (3.13) and (3.14) -Benzotriazoles[a]

| Comp. | Bt-signals | | | | | | α-$CH_2$ | β-$CH_2$ |
|---|---|---|---|---|---|---|---|---|
| No. | C-3a | C-4 | C-5 | C-6 | C-7 | C-7a | | |
| 3.5 | 144.9 | 118.8 | 125.2 | 127.1 | 109.8 | 133.4 | 60.8 | 50.6 |
| 3.6 | 145.7 | 119.9 | 124.0 | 127.6 | 109.3 | 133.4 | 49.3 | 42.2 |
| 3.13 | 145.5 | 119.8 | 123.9 | 127.5 | 109.1 | 133.0 | 49.1 | 28.8 |
| 3.7 | 144.4 | 118.1 | 126.8 | - | - | - | 61.1 | 58.9 |
| 3.8 | 144.4 | 118.0 | 126.5 | - | - | - | 57.3 | 41.4 |
| 3.14 | 144.4 | 117.9 | 126.6 | - | - | - | 57.2 | 28.1 |

[a] In $CDCl_3$ with $Me_4Si$ as reference.

Table 3.2. $^{1}H$ N.m.r. Chemical Shifts (δ) of β-Hydroxyethyl- (3.5) and (3.7) and β-Haloethyl- (3.6), (3.8), (3.13) and (3.14) -Benzotriazoles[a]

| Comp. | Bt- | α-$CH_2$ | β-$CH_2$ |
|---|---|---|---|
| No. | (m, 4 H) | | |
| 3.5 | 7.2-8.0 | 4.5-4.8 (m)[b] | 4.3 (t, J= 7Hz) |
| 3.6 | 7.2-8.2 | 5.1 (t, J=7 Hz) | 4.1 (t, J= 7Hz) |
| 3.13 | 7.2-8.2 | 5.0 (t, J=7 Hz) | 3.8 (t, J= 7Hz) |
| 3.7 | 7.3-7.7 | 4.8 (t, J=7 Hz) | 4.3 (m)[b] |
| 3.8 | 7.7-8.2 | 5.1 (t, J=7 Hz) | 4.2 (t, J= 7Hz) |
| 3.14 | 7.3-8.3 | 5.2 (t, J=7 Hz) | 4.2 (t, J= 7Hz) |

[a] In $CDCl_3$ with $Me_4Si$ as reference.

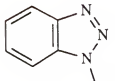
[b] Signal overlaps with that of the -OH group.

The resonance of the carbon α to benzotriazole (Table 3.1) appeared between 49.1-61.1 ppm, with a difference of about 8 ppm for the 1- and 2-isomers of both chloro and bromo derivatives. The resonance of the carbon β to benzotriazole was in the range between 28.1-58.9 ppm. In the hydroxy derivatives, the β carbon atoms are more deshielded than that of the chloro and bromo derivatives. A difference of about 14 ppm between chloro and bromo derivatives in the β carbon resonances was also observed which could be attributed to the difference in their electronegativity.

The $^{1}H$ n.m.r. spectra (Table 3.2) showed the expected ratio of protons, multiplicity pattern and reasonable chemical shifts for the benzotriazole ring as well as for the α and β protons. Unlike the $^{13}C$ n.m.r., there is not a remarkable difference between the 1- and 2- isomers in the chemical shift of α and β protons. Very little difference is observed in the chemical shifts of β protons between hydroxy and halo derivatives.

### 3.3.2. Nucleophilic Displacement on β-Chloroethylbenzotriazole (3.6)

3.3.2.1. Sulfur nucleophile



(3.9a) $CH_2CH_2S(CH_2)_7CH_3$
α β

An equimolar quantities of sodium ethoxide, n-octylthiol, and chloroethylbenzotriazole (3.6) in ethanol under refluxing conditions for one hour resulted in mainly the nucleophilic displacement of the chlorine atom by a sulfur anion affording 2-(benzotriazol-1-yl)ethyl n-octyl sulfide (3.9a). About 10% of elimination product, 1-vinylbenzotriazole (3.12)was also formed. A change in base from sodium ethoxide to sodium hydroxide under the above conditions afforded 94% of (3.9a). The compound (3.9a) was characterized by its carbon and proton n.m.r. data and its structure was further supported by elemental analysis and a high resolution mass spectrum. The salient pattern of a 1-substituted benzotriazole was observed in addition to the ten alkyl carbon resonances (Table 3.3). The carbon resonance β to the benzotriazole group is shielded due to sulfur atom by about 10 ppm as compared to the starting material (3.6). The proton n.m.r. also showed a shielding of the β-protons by about 1 ppm from the starting material.

Table 3.3 $^{13}C$ N.m.r. Chemical Shifts (δ) of β-Heteroatom Linked Ethylbenzotriazoles (3.9a-d)[a]

| Comp. | Bt-signals | | | | | | α-$CH_2$ | β-$CH_2$ |
|---|---|---|---|---|---|---|---|---|
| No. | C-3a | C-4 | C-5 | C-6 | C-7 | C-7a | | |
| 3.9a[b] | 146.2 | 120.0 | 123.9 | 127.3 | 109.2 | 135.2 | 48.2 | 32.4 |
| 3.9b[c] | 145.2 | 119.2 | 123.0 | 126.3 | 108.9 | 132.7 | 53.9 | 53.0 |
| 3.9c[d] | 145.7 | 119.6 | 123.9 | 127.6 | 107.9 | 133.1 | 47.5 | -[d] |
| 3.9d | 144.3 | 119.7 | 123.7 | 127.3 | 108.4 | 132.9 | 47.0 | 55.2[e] |
| | 144.4 | 117.7 | 126.5 | - | - | - | - | - |

[a] In $CDCl_3$.

[b] S-octyl = 14.0, 22.6, 28.7, 29.0, 29.1, 29.5, 31.6, 31.7.

[c] N-$(octyl)_2$ = 13.4, 21.9, 26.6, 28.5, 28.6, 28.8, 31.1, 46.5.

[d] (1,1)-isomer - symmetrical compound.

[e] Carbon attached to 2-benzotriazolyl.

The preceding discussion gives an idea of the leaving group ability of the benzotriazole and chloride anions. Although there is an equal statistical chance in this ethyl derivative (3.6), only the chloride ion is displaced but not the benzotriazole group. Similar results are also obtained for chloromethyl derivatives with various nucleophiles [87JCS(P1)781]. This may be explained as follows: good leaving groups are the conjugate bases of strong acids. The pKa of an acid reflects its strength; the pKa for HCl is <0 and for benzotriazole is approximately 8.3. Hence chloride anion is a better leaving group than benzotriazole anion. The mechanism of the reaction is likely to be $SN_2$.

Table 3.4. $^1$H N.m.r. Chemical Shifts (δ) of β-Heteroatom Linked Ethylbenzotriazoles (3.9a-d)[a]

| Comp. No. | Bt- | α-$CH_2$ | β-$CH_2$ |
|---|---|---|---|
| 3.9a[b] | 7.4-8.5 (m, 4H) | 4.9 (t, J=7 Hz) | 3.3 (t, J=7 Hz) |
| 3.9b[c] | 7.3-8.4 (m, 4H) | 4.8 (t, J=7 Hz) | 3.1 (t, J=7 Hz) |
| 3.9c[d] | 6.7-7.5 (m, 6H) | 5.3 (s, 4H) | -[d] |
| | 7.8-8.2 (m, 2H) | | |
| 3.9d[e] | 7.1-7.7 (m, 5H) | 5.2-5.7 (bs, 4H) | -[e] |
| | 7.7-8.4 (m, 3H) | | |

[a] In $CDCl_3$.

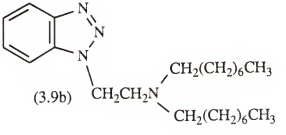
[b] -S-octyl = 1.0 (t, J=7 Hz, 3H), 1.2-2.3 (m, 12H), 2.6 (t, J=7 Hz, 2H)

[c] $-N(octyl)_2$ = 0.2-1.8 (m, 30H), 2.0-2.9 (m, 4H).

[d] 1,1-Isomer.

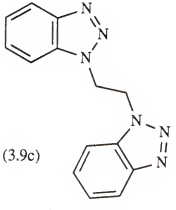
[e] 1,2-Isomer.

3.3.2.2. Nitrogen nucleophile

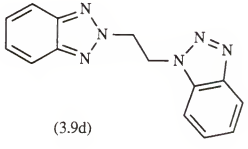



(3.9b)

An attempted synthesis of 2-(benzotriazol-1-yl)ethyl dioctylamine (3.9b) from (3.6) and dioctylamine, in a molar ratio of 1:2 in toluene under refluxing conditions, gave only vinyl benzotriazole (3.12). When a catalytic amount of sodium iodide was added a small amount of (3.9b) along with vinylbenzotriazole (3.12) was obtained. Adopting the same method as that for the sulfide (3.9a) was also unsuccessful giving only vinylbenzotriazole (3.12). Hence, one equivalent of sodium iodide, one equivalent of (3.6), and two equivalents of dioctylamine were heated between 120-130°C for two hours to afford (3.9b) in 30% yield. $^{13}C$ (Table 3.3) and $^{1}H$ n.m.r. (Table 3.4) data provided evidence for the structure, which was further supported by elemental analysis. The chemical shifts in $^{13}C$ and $^{1}H$ n.m.r. for all the carbons and protons are similar to the n-octylthiol derivative (3.9a) except for the chemical shift of the α and β carbon signals of (3.9b) (Table 3.3).



(3.9c)



(3.9d)

The benzotriazole anion itself is a good nitrogen nucleophile and so can be used to prepare 1,2-bis(benzotriazolyl)ethanes (3.9c) and (3.9d). Synthesis of 1,2-bis(benzotriazolyl)ethanes had been reported by a three step sequence starting from benzotriazole via (3.5) and (3.6) [38CB596]. It has also been obtained from two equivalents of ethylenebromide and one equivalent of the sodium salt of benzotriazole in 42% yield [38CB596]. Attempts on improving the later method, were made and the optimum conditions were determined to be using the same molar ratio of the starting materials on prolonged heating at 100°C for three days. The product was a mixture of 1,2-bis(benzotriazol-1-yl)ethane (3.9c) and 1,2-bis(benzotriazol-1- and 2-yl)ethane (3.9d) in an overall yield of 83%. The two isomers were separated by crystallization from dioxane for (3.9c), and from methanol for (3.9d). Their melting points agreed with literature melting points [38CB596]. In addition, $^{13}C$ and $^{1}H$ n.m.r. data also provided evidence for their structures (Tables 3.3 and 3.4). The symmetry of (3.9c) is seen both in benzotriazole carbons and in the alkyl carbons, showing only seven resonances altogether. For (3.9d) the dissymmetry is observed in the carbon signals. The alkyl protons were still observed as a singlet, although broader than that for the other isomer (3.9c).

## 3.4. Conclusions

Successful nucleophilic displacement on chloroethyl benzotriazole (3.6) by sulfur and nitrogen nucleophiles has been achieved to afford β-heteroatom linked ethylbenzotriazoles, (3.9a-d). The less successful attempts leading to the elimination product, vinylbenzotriazole (3.12), opens a new possibility of using chloroethylbenzotriazole (3.6) as a protecting group similar to vinylpyridines.

For the previously known compounds, conditions were optimized to get higher yields, and $^{13}C$ and $^{1}H$ spectral data were discussed.

## 3.5. Experimental

Melting points were determined on a Bristoline hot-stage microscope and are uncorrected. $^{1}H$ n.m.r. spectra were recorded on a Varian EM 360L or XL-200 spectrometer with tetramethylsilane as an internal reference. $^{13}C$ n.m.r. spectra were recorded on a Varian XL-200 (50 MHz) spectrometer referring to the middle signal of $CDCl_3$ (77.0 ppm). Elemental analyses were performed under the supervision of Dr. D. H. Powel and Dr. R. W. King at the University of Florida Department of Chemistry.

3.5.1. Synthesis of β-Hydroxyethylbenzotriazoles (3.5) and (3.7)

To a solution of benzotriazole (47.7 g, 0.4 mol) in aqueous sodium hydroxide (2N, 220 mL), 2-chloroethanol (26.8 mL, 0.4 mol) was added in drops. The mixture was heated at 100°C for 2 h. A colorless oil or a brown oil was obtained depending upon the purity of the benzotriazole. The mixture was extracted with ether, and the ether extract was dried with magnesium sulfate. The 1-isomer (3.5) crystallized from the ether solution and a repeated harvest of the white needles left the 2-isomer in the mother liquor which subsequently crystallized as colorless rectangular crystals. The yield of the 1-isomer was 57% (34 g) and that of the 2-isomer was 20% (12 g). The melting point of the 1-isomer (3.5) was 90-91°C, lit. m.p. 90-91°C [38CB596]; and that of the 2-isomer (3.7) was 69-71°C, lit. m.p. 70-71°C [38CB596]. $^{13}C$ and $^{1}H$ n.m.r. data are given in Tables 3.1 and 3.2, respectively.

3.5.2. Synthesis of β-Chloroethylbenzotriazoles (3.6) and (3.8)

To a cold solution of β-hydroxyethylbenzotriazole (3.5) (25 g, 0.153 mol) in chloroform (150 mL) at 0°C, thionyl chloride (16.7 mL, 0.229 mol) was added in drops. The mixture was kept stirring at room temperature for 6 h. After this time the solution was refluxed for 2 h, and the

removal of solvent under reduced pressure yielded (3.6) as a white solid. Recrystallization from benzene gave white needles (22.5 g, 81%), m.p. 108-110°C, lit. m.p. 108-109°C [57MI904].

β-Hydroxyethylbenzotriazole (3.7) (25g, 0.153 mol) and thionyl chloride (16.7 mL, 0.229 mol) in chloroform (150 mL) under the same experimental conditions as for (3.6) gave 88% of (3.8) as a white solid. Recrystallization from benzene gave white needles, m.p. 38-40°C. Calculated for $C_8H_8ClN_3$; Required C, 52.90; H, 4.44; N, 23.14; Found C, 53.07; H, 4.33; N, 23.42. $^{13}C$ and $^{1}H$ n.m.r. data are given in Tables 3.3 and 3.4, respectively.

### 3.5.3. Synthesis of β-Bromoethylbenzotriazoles (3.13) and (3.14)

To a solution of benzotriazole (4.77 g, 40 mmol) in aqueous sodium hydroxide (2N, 22 mL), ethylenebromide (14.96 g, 80 mmol) was added and heated to 80-85°C for 2 h. The reaction mixture was extracted with ether, and the ether extract was dried with $MgSO_4$. On cooling the 1-isomer (3.13) precipitated as a white fluffy solid. This was filtered and washed with cold ether to give the 1-isomer (3.13) (2.25 g, 33%), m.p. 108-109°C; lit. m.p. 109-110°C [38CB596]. The mother liquor contained 2-isomer (3.14) and a small amount of the 1-isomer (3.13). Repetitive

recrystallization using diethyl ether and petroleum ether gave the pure 2-isomer (3.14) (yield 25%), m.p. 59-62°C, lit. m.p. 59-60°C [38CB596].

### 3.5.4. Synthesis of 2-(Benzotriazol-1-yl)ethyl n-Octyl Sulfide (3.9a)

To a solution of n-octylthiol (0.86 mL, 5 mmol) in 95% ethanol (5 mL) was added an aqueous solution of sodium hydroxide (10%, 2 mL). This mixture was added to a solution of β-chloroethylbenzotriazole (3.6) (0.91 g, 5 mmol) in 95% ethanol (5 mL). The whole mixture was refluxed for 1 h, and the hot solution was then filtered to remove sodium chloride. Removal of the solvent under reduced pressure afforded (3.9a) as a white solid. Recrystallization from MeOH or EtOH gave white flakes (1.37 g, 94% yield), m.p. 41-43°C. Calculated for $C_{16}H_{25}N_3S$, Required C, 65.94; H, 8.65; N, 14.42; Found C, 65.36; H, 8.70; N, 14.24. HR/MS Calculated: $M^+$ 291.17692; Found: 291.17539. $^{13}C$ n.m.r. δ ($CDCl_3$) 14.0, 22.6, 28.7, 29.0, 29.1, 29.5, 31.6, 31.7, 32.4, 48.2, 109.2, 120.0, 123.9, 127.3, 135.2, 146.2. $^1H$ n.m.r. δ ($CDCl_3$) 1.0 (t, J=7Hz, 3H), 1.2-2.3 (m, 12H), 2.6 (t, J=7Hz, 2H), 3.3 (t, J=7Hz, 2H), 4.9 (t, J=7Hz, 2H), 7.4-8.5 (m, 4H).

### 3.5.5. Synthesis of 2-(Benzotriazol-1-yl)ethyldioctylamine (3.9b)

β-Chlorethylbenzotriazole (0.91 g, 5 mmol), sodium iodide (5 mmol) and dioctylamine (2.41 g, 10 mmole) were heated between 120-130°C for 2 h. The reaction mixture turned into a brown solid on cooling to room temperature. Washing with ether gave a white solid which was recrystallized from petroleum ether to give (3.9b) in 30% yield, m.p. 45-48°C. Calculated for $C_{24}H_{42}N_4$, Required C, 74.56; H, 10.95; N, 14.49; Found C, 74.13; H, 11.08; N, 14.41. $^{13}C$ n.m.r. δ ($CDCl_3$) 13.4, 21.9, 26.6, 28.5, 28.6, 28.8, 31.1, 46.5, 53.0, 53.9, 108.9, 119.2, 123.0, 126.3, 132.7, 145.2. $^1H$ n.m.r. δ ($CDCl_3$) 0.2-1.8 (m, 30H), 2.0-2.9 (m, 4H), 3.1 (t, J=7Hz, 2H), 4.8 (t, J=7Hz, 2H), 7.3-8.4 (m, 4H).

### 3.5.6. Synthesis of 1,2-Bis-(benzotriazol-1-yl)ethane (3.9c) and 1-(Benzotriazol-1-yl)-2-(benzotriazol-2-yl)ethane (3.9d)

To a solution of benzotriazole (38.16 g, 0.32 mol) in aqueous sodium hydroxide solution (2N, 176 mL), 1,2-dibromoethane (29.92 g, 0.16 mol) was added slowly at room temperature. Heating at 100°C for 3 days gave a pale yellow oil. Addition of ether to the reaction mixture yielded a pale yellow granular solid. The solid was filtered and the filtrate was further extracted with ether. From the ether layer additional pale yellow solid was obtained. The overall yield was 83%. Recrystallization from dioxane, gave

1,2-bis(benzotriazol-1-yl)ethane (3.9c) as white crystals, m.p. 159-162°C, lit. m.p. 161-162°C [38CB596]. Recrystallization from methanol gave 1-(benzotraizol-1-yl)-2-(benzotriazol-2-yl)ethane (3.9d) as white needles, m.p. 135-137°C, lit. m.p. 136-137°C [38CB596]. $^{13}C$ and $^{1}H$ n.m.r. data are given in Tables 3.3 and 3.4, respectively.

CHAPTER 4

SYNTHESIS OF α-ACTIVATED ALKYL ISOCYANIDES

## 4.1. Introduction

Although isocyanides have been known for a century, systematic investigation did not begin until about 1960. Little detailed attention has been given to mechanistic studies of isocyanide reactions, although they exhibit unique possibilities. Apart from the isomerization of isocyanide to cyanide [87JOC648], there is a very little quantitative data available concerning isocyanide chemistry. The main reason is probably the lack of access to pure isocyanides in high yield, and may also be due to their renowned malodorous property which was described by the discoverers Hofmann and Gauiter as "highly specific and almost overpowering," "horrible" and "extremely distressing" [65AG(E)472].

### 4.1.1. Structure of the Isocyanide Group


(4.1) (4.2)

Scheme 4.1

The isocyano group is unique because it is the only stable functional group with a bivalent carbon atom. It is represented in two resonance forms (Scheme 4.1) in which (4.1) illustrates the carbenic character and (4.2) illustrates the potential nucleophilic character. The pronounced ability of an isocyanide, is that it can be easily introduced into other molecules. This is due to the unique combination of unsaturation, polarization, and low steric demand similar to nitriles, and to the peculiar isocyanide chemistry due to the formal divalency of the isocyanide carbon.

### 4.1.2. Physical and Chemical Properties of Alkyl Isocyanides

The electronic structures of a few simple alkyl isocyanides have recently been calculated. Using Huckel [68JAM3227], MNDO [72JAM2704] and ab initio [80JAM3719] calculations, the geometrical configuration of methyl isocyanide has been been determined (Figure 4.1).

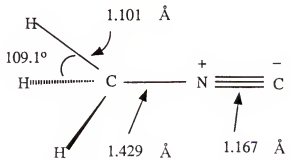
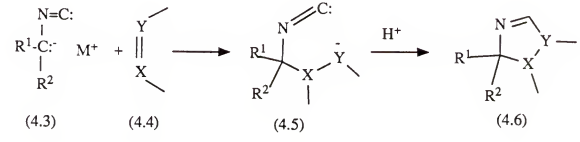



Figure 4.1 Bond Lengths and Bond Angles in Methyl Isocyanide.

The isocyanide and cyanide groups are similar in some respects but strikingly different in others. Calculation of electron densities [58JMS54] using the nuclear positions and dipole moments ($\mu$ = 3.92 D for $CH_3CN$ and $\mu$ = 3.83 D for $CH_3NC$) suggest similar electron distribution between the carbon and the nitrogen ($Q_C$ 0.533 and $Q_N$ 1.475) of the isocyanide group and of the cyanide group. By contrast, mass spectral data of some aliphatic isocyanides and the corresponding cyanides indicate differences in the fragmentation pathways [63JOC2924]. α-cleavage is prominent in isocyanides, while it is of minor importance with cyanides. The absorption frequency of the N≡C: stretch in the infrared spectrum of an isocyanide is approximately 100 $cm^{-1}$ lower than the corresponding CN triple bond stretching frequency for a cyanide.

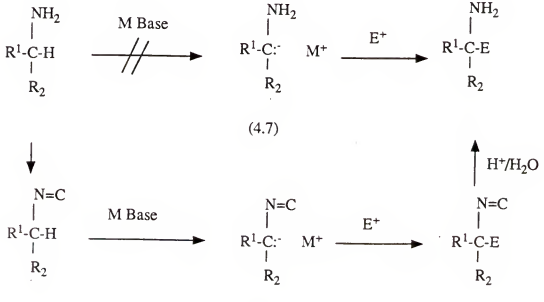



(4.3) (4.4) (4.5) (4.6)

Scheme 4.2

A wide range of chemical reactions of isocyanides, such as simple α-addition , cyclization, peptide synthesis, polymer synthesis, and Passerini reaction can be found in a

number of interesting reviews [62AG(E)8, 72CR101, 74AG(E)789, 82AG(E)810]. Isocyanides containing α-hydrogen atoms react with strong bases by α-deprotonation giving metallated isocyanides. These have been well exploited as versatile synthetic intermediates. The significance of α-metallated isocyanides (4.3) can be attributed to the ambivalent nature of these reagents. They (4.3) contain a nucleophilic center (the anionic carbon) which can add to a polar multiple bond (4.4), and an electrophilic centre (the isocyanide carbon atom) which permits cyclization of the adducts (4.5) to form heterocycles of type (4.6) (Scheme 4.2). On the other hand, α-metallated isocyanides (4.3) are masked α-metallated primary amines (4.7) and permit chain extension, or elaboration of primary amines by electrophiles (Scheme 4.3).




Scheme 4.3

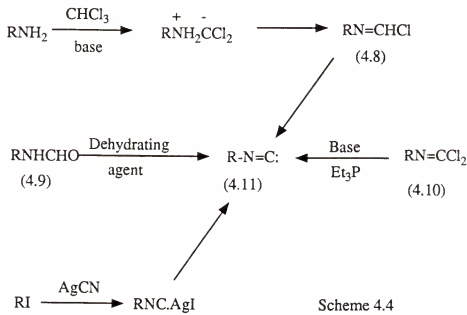
The reactivity of an isocyanide with an α-hydrogen may be further enhanced by an activating group in the α-position. If this activating group is also a good leaving group, the reactions in schemes 4.2 and 4.3 become more versatile. One such system which is well exploited in organic synthesis is van Leusen's toluene-p-sulfonyl isocyanide (TOSMIC) [77JOC1153, 77JOC3114, 79MI2857, 82MI4119].

4.1.3. Benzotriazole as an Activating and a Leaving Group

The introduction of benzotriazole at the α-position of an alkyl isocyanide has several advantages. More alkyl or aryl substituents can be at the α-position, and in addition, the benzotriazole group can activate the methylene (or methine) group as well as function as a good leaving group. Activation of the methylene group is due to the strong electron withdrawing ability of the N=N double bond of benzotriazole, and this feature is reflected in its pKa of 8.38 [88JAM4105]. The relative acidic nature of benzotriazole also enables easy separation and recovery, and benzotriazole can be cleaved or replaced by a variety of procedures. These two properties of benzotriazole have been extensively studied by Katritzky et. al. [87JCS(P1)781]. Furthermore benzotriazole is readily available and inexpensive.

## 4.1.4. Synthesis of Alkyl Isocyanides

Most of the reactions on which isocyanide synthetic methods are based were discovered only in the last decade.



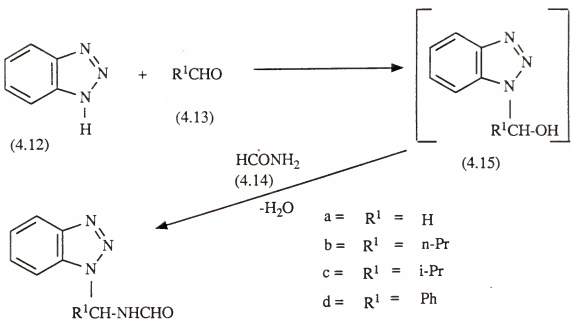

Scheme 4.4

The synthesis of isocyanides (4.11) (Scheme 4.4) is invariably accompanied by conversion of a tetravalent carbon atom into a divalent carbon atom via elimination or redox reaction. The classical isocyanide (4.11) synthesis - the "carbamyl (4.8) reaction" of Hofmann [1867A(144)114], and the synthesis of isocyanides by the alkylation of silver cyanide according to Lieke and Gautier [1867A(142)289], are of little use for the preparation of pure isocyanides. The synthesis of isocyanides (4.11) from N-monosubstituted formamides (4.9) only proceeds well with an acid forming dehydrating agent, such as arenesulfonyl chlorides

[58JOC1221, 72JOC187], phosphorus oxychloride [60CB239], cyanuric chloride [61AG219], phosgene [61CB2814], or diphosgene [77AG(E)259]. An excess of tertiary amine is most often used to promote the α,α-elimination from the imine chloride intermediate (4.10) and to consume the hydrogen halide which is generated. Methylene chloride and other halogenated hydrocarbons are the common solvents of choice. There have been various improvements [85CC400, 71AG(E)132, 72TL1637, 77AG(E)262, 78AG(E)688, 77AG(E)259] to these methods, and phosphorus oxychloride, in combination with di-isopropyl amine in methylene chloride, seems to be a mild and general method giving good yields with high purity [85CC400].

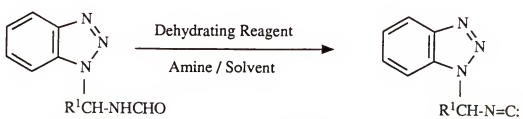
## 4.2. Objectives

The objective of this work is to develop a general method of preparing alkyl isocyanides with benzotriazole α-activation via N-monosubsituted formamides. Previously, Katritzky and coworkers [88JCS(P1)2339] have shown that benzotriazole reacts with an aldehyde and an alkyl- or aryl-amide to form a 1:1:1 adduct; a N-monosubstituted alkyl- or aryl-amide. Similarly benzotriazole (4.12), an aldehyde (4.13), and formamide (4.14) could be expected to yield 1:1:1 adducts; N-monosubstituted formamides (4.16) (Scheme 4.5).




Scheme 4.5

The corresponding adducts, that is the N-monosubstituted formamides (4.16 a-d), with a suitable dehydrating reagent, an amine, and solvent should give alkyl isocyanides (4.17 a-d) with α-activation by benzotriazole (Scheme 4.6).




Scheme 4.6

These isocyanides (4.17 a-d) should not be malodorous since they are of high molecular weight. If successful, α-metallation followed by electrophilic substitution (Scheme 4.7) will further modify the α-carbon leading to (4.19). In addition, modification at the isocyano divalent carbon is possible at virtually any stage.


(4.17) (4.18) (4.19)

Scheme 4.7

## 4.3. Results and Discussion

### 4.3.1. Synthesis of N-(α-Benzotriazol-1-yl)alkylformamides (4.16 a-d)

N-(α-Benzotriazol-1-ylalkyl)formamides (4.16) were synthesized in good yields from benzotriazole (4.12), the corresponding aldehyde (4.13), and formamide (4.14) in a one pot reaction in toluene under refluxing conditions. Water, the side product formed, was removed azeotropically by a Dean Stark trap. Attempts with both aliphatic and aromatic

aldehydes were successful (Scheme 4.5) (Table 4.1). Formation of N-(α-benzotriazol-1-yl)alkylformamides (4.16) could have also followed the same synthetic route, that is an extended Mannich reaction via the hydroxyalkylbenzotriazole intermediate (4.15) (Scheme 4.5) [87JCS(P1)799, 88JCS(P1)2339].

Table 4.1 Synthesis of N-(α-Benzotriazol-1-yl)alkyl-formamides (4.16 a-d).

| Comp. No. | Yield (%) | M.p. (°C) | Formula | Analysis (%) Required (Found) | | |
|---|---|---|---|---|---|---|
| | | | | C | H | N |
| 4.16a | 63[a] | 138-139[b] | $C_8H_8N_4O$ | 54.5 | 4.6 | 31.8 |
| | | | | (54.2) | (4.4) | (32.0) |
| 4.16b | 87 | oil[c] | $C_{11}H_{14}N_4O$ | [d] | | |
| 4.16c | 92[e] | 93-95 | $C_{11}H_{14}N_4O$ | 60.5 | 6.5 | 25.7 |
| | | | | (60.4) | (6.0) | (25.8) |
| 4.16d | 77[a] | 141-142 | $C_{14}H_{12}N_4O$ | 66.7 | 4.8 | 22.2 |
| | | | | (66.7) | (4.7) | (22.4) |

[a] Crystallized from ethanol.

[b] Lit. m.p. 146-147°C [83MI723].

[c] B.p. 95-100°C/0.5 mm Hg.

[d] Calculated mass 218.11676, found (HR/MS) 218.11682.

[e] Crystallized from hexane/ether.

Carbon and proton nuclear magnetic resonance (n.m.r.) and infrared spectral (i.r.) results gave evidence for the structures which were further confirmed by elemental analysis or high resolution mass spectra (HR/MS). Assignment of δ values benzotriazole ring carbon atoms (Table 4.2) was done by reference to previously reported 1-substituted benzotriazoles [83H1787]. The typical $^{13}C$ pattern of 1-substituted benzotriazole derivatives were observed in all adducts (Table 4.2). The carbonyl carbon resonance appeared between 159.6 - 161.9 ppm and, as expected it varied little with the variation in N-substitution [80OMR126]. The methylene carbon between N-1 of benzotriazole and the nitrogen atom of the amide resonated at 48.2 ppm in (4.16a). When there was an alkyl (4.16 b and c) or aryl (4.16d) substituent the methine carbon appeared between 61.0 - 65.9 ppm. The number of carbon signals for alkyl and aryl substituents were as expected, three for n-propyl and four for phenyl but three for i-propyl revealing the non-equivalency of the methyl carbons.

Table 4.2 $^{13}C$ N.m.r. Chemical Shifts (δ) of N-(α-Benzotriazol-1-yl)alkylformamides (4.16a-d).[a]

| Comp. | Bt signals | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| No. | C-3a | C-4 | C-5 | C-6 | C-7 | C-7a | Bt-CH | CHO |
| 4.16a | 144.6 | 118.1 | 123.0 | 126.4 | 109.8 | 131.1 | 48.2 | 160.8 |
| 4.16b | 146.0 | 120.0 | 125.0 | 128.4 | 110.9 | 133.3 | 61.0 | 161.9 |
| 4.16c | 145.2 | 124.4 | 119.5 | 127.9 | 110.0 | 133.2 | 65.9 | 161.4 |
| 4.16d | 144.2 | 118.0 | 122.6 | 126.1 | 108.9 | 130.6 | 61.8 | 159.6 |

[a] In $CDCl_3$/DMSO-$d_6$ (for 4.16 a and d) and $CDCl_3$ (for 4.16 b and c) with $Me_4Si$ as the reference, and the $R^1$ signals are at 13.8, 19.2 and 36.3 for (4.16b), 18.6, 18.8 and 32.7 for (4.16c) and 124.9, 127.2, 124.4 and 134.2 for (4.16d).

The $^1H$ n.m.r. spectra (Table 4.3) showed the typical pattern of 1-substituted benzotriazole, that is H-4 of benzotriazole more shielded than the other three protons (H-5, H-6 and H-7) in all adducts except in (4.16d) in which the phenyl protons overlapped with benzotriazole and gave a complex pattern. The methylene protons in (4.16a) appeared at 6.2 ppm and alkyl substitution at this carbon did not significantly affect the methine protons chemical shift, that is 6.9 and 6.5 ppm in (4.16b) and (4.16c), respectively. With an aryl substituent (4.16d) the methine proton is more deshielded and appears at 8.3 ppm. The

multiplicity pattern of the methylene and methine protons of these adducts (4.16 a-d) were as expected. This helped to identify their structure as well as to follow the reaction in the subsequent step. Among the proton signals of substituents the two non-equivalent methyl groups were seen as two doublets at 0.7 and 1.2 ppm, for (4.16c). N-H Protons resonated between 9.2 - 10.2 ppm as a doublet with a coupling constant range of 8-9 Hz except in (4.16a) where it appeared as a broad singlet. The formyl proton appeared between 8.2-8.6 ppm as a singlet in all adducts (4.16 a-d).

Table 4.3 $^1H$ N.m.r. Chemical Shifts (δ) of N-(α-Benzotriazol-1-yl)alkylformamides (4.16 a-d).[a]

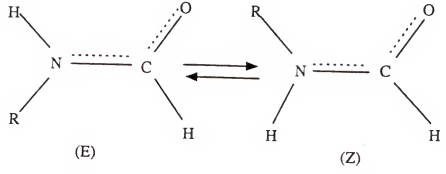
| Comp. No. | Bt | Bt-C<u>H</u>(R) (m, J(Hz), H) | CHO (s, 1H) | NH (m, J(Hz), 1H) |
|---|---|---|---|---|
| 4.16a | 7.2-8.2 (m, 4H) | 6.2 (d, 7, 2) | 8.2 | 9.3 (bs) |
| 4.16b | 7.2-8.4 (m, 4H) | 6.9 (m, 1) | 8.5 | 9.3 (d, 7) |
| 4.16c | 7.2-8.4 (m, 5H)[b] | 6.5 (t, 9, 1) | 8.6 | _[b] |
| 4.16d | 7.6-8.2 (m, 5H) | _[c] | 8.6 | 10.2 (d, 9) |

a In $CDCl_3$/DMSO-$d_6$ (for 4.16 a and d) and $CDCl_3$ (for 4.16 b and c) with $Me_4Si$ as the reference, and the signals for $R^1$ groups are, 0.9 (t, J = 7Hz, 3H), 1.1-1.8 (m, 2H) and 2.5 (q, J = 7Hz, 2H) for (4.16b), 0.7 (d, J = 7Hz, 3H), 1.2 (d, J = 7Hz, 3H) and 2.2-3.2 (m, 1H) for (4.16c) and 7.5 (s, 5H) for (4.16d).

b NH signal overlaps with benzotriazole signals.

c Bt-C<u>H</u>-R signal overlaps with the benzotriazole signals.

From the $^{13}C$ and $^{1}H$ n.m.r. spectral data it appears that the N-monosubstituted formamides most likely exist in the Z form. There are only one set of peaks, and the coupling constant of NH with CHO is less than 1 Hz. In addition, the chemical shift of the carbonyl carbon is between 160 - 162 ppm which is very close with the Z form of methyl formamide (162.8 ppm) [80OMR126], [70CR517]. These data however, do not rule out the possibility of rapid rotation around the C-N bond (Scheme 4.8) or other factors that may cause chemical shift degeneracy of the cis and trans signals.




Scheme 4.8

The other inference is that there are no 1- and 2-isomerizations of benzotriazole observed in these adducts (from their n.m.r. spectra).

An i.r. spectrum of these compounds showed the characteristic secondary amide carbonyl stretch in the range 1678 - 1682 $cm^{-1}$, and an NH stretch at 3225 - 3260 $cm^{-1}$. The carbonyl stretch frequency is an useful tool for

following the reaction of these adducts in the subsequent step.

### 4.3.2. Synthesis of N-(α-Benzotriazol-1-yl)alkyl Isocyanides (4.17 a-d)

Although the dehydration of an N-monosubstituted formamide to afford an isocyanide is the most applicable general method, the choice of the right dehydrating reagent, amine, and solvent for the system with benzotriazole and various substituents required persistent and persevering attempts.

Using phosphorus oxychloride, and triethylamine in methylene chloride at 0°C for 1 h gave (4.17a) in 66% yield. Application of the same method for (4.17b) and (4.17c) was less successful. Four spots were observed with t.l.c. (including the starting material) indicating an incomplete reaction. Hence, other existing procedures such as p-toluenesulfonylchloride [58JOC1221], or thionyl chloride and pyridine [72JOC187] or triphenylphosphine and carbon tetrachloride [71AG(E)132] were adopted but also proved to be unsuccessful. Phosphorus oxychloride and di-isopropylamine in methylene chloride at 0°C for 1 - 2 h were determined to be the optimum conditions. The isocyanides (4.17 a-d) were isolated in good yields (Table 4.4). Scaling up to 0.5 mole was also successful. Isocyanides (4.17a) and (4.17d) do not have the malodor unlike (4.17b) and (4.17c).

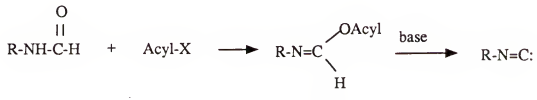
Table 4.4 Synthesis of N-(α-Benzotriazol-1-yl)alkyl Isocyanides (4.17 a-d).

| Comp. No. | Yield (%) | M.p. (°C) | Formula | Molecular Mass Required | Found (HRMS) |
|---|---|---|---|---|---|
| 4.17a | 66[a] | 104-106[b] | $C_8H_6N_4$ | - | - |
| 4.17b | 64 | oil | $C_{11}H_{12}N_4$ | 200.10620 | 200.10666 |
| 4.17c | 72 | 25-28 | $C_{11}H_{12}N_4$ | 200.10620 | 200.10663 |
| 4.17d | 77 | oil | $C_{14}H_{10}N_4$ | 234.09055 | 234.09012 |

[a] Crystallized from benzene.

[b] Lit., m.p. 103-106 [83MI723].

The reaction of N-monsubstituted formamides with acylating agents and bases proceeded in two steps: the base catalyzed O-acylation to give (4.20), followed by the nucleophilic α-elimination of a proton and an acid anion to give the isocyanides (Scheme 4.9) [65AG(E)472].




(4.20)

Scheme 4.9

The isocyanides were characterized by i.r., $^{13}C$ and $^{1}H$ n.m.r. spectral data and were confirmed by HR/MS, or melting point from literature (for 4.17a).

In the i.r. spectra the characteristic isocyanide N=C: stretching absorption was at 2132 $cm^{-1}$ for all four isocyanides (4.17 a-d), hence the substitution did not cause any effect. However, the intensity of the peak was very strong when there was a phenyl substituent which inductively stabilizes the canonical form (4.2). Generally the greater intensity of the isocyanide band to that of the corresponding nitrile is due to the greater charge separation as shown in (4.2) [69MI7].

Table 4.5 $^{13}C$ N.m.r. Chemical Shifts (δ) of N-(α-Benzotriazol-1-yl)alkyl Isocyanides (4.17 a-d).[a]

| Comp. | Bt signals | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| No. | C-3a | C-4 | C-5 | C-6 | C-7 | C-7a | Bt-CH | N=C |
| 4.17a | 146.1 | 120.4 | 124.9 | 128.9 | 108.8 | 131.7 | 52.3 | 163.3 |
| 4.17b | 147.3 | 120.4 | 124.7 | 128.4 | 109.6 | 131.3 | 66.3 | 162.3 |
| 4.17c | 146.7 | 120.9 | 125.2 | 128.9 | 110.3 | 131.5 | 72.9 | 163.5 |
| 4.17d | 147.1 | 120.9 | 125.2 | 128.9 | 110.7 | 131.1 | 69.3 | 164.8 |

[a] In $CDCl_3$ with $Me_4Si$ as internal reference, and the $R^1$ groups showed resonances at 12.7, 18.0 and 36.7 for (4.17b), 18.1, 19.0 and 34.5 for (4.17c) and 126.1, 129.7, 124.8 and 130.8 for (4.17d).

In the $^{13}C$ n.m.r. spectra of isocyanides (4.17 a-d) (Table 4.5), the six signals for the benzotriazole ring carbon atoms showed the typical pattern of 1-substituted benzotriazole. All six signals however, were deshielded about 2 ppm from the N-monosubstituted formamide precursors (4.16 a-d). The methylene carbon or methine carbon between the benzotriazole N-1 atom and the nitrogen atom of the isocyano group resonated between 52.3 - 69.3 ppm which is a remarkable deshielding of about 4.5 ppm. The isocyano carbon resonance appeared in the range of 162.3 - 164.8 ppm. The intensity of the isocyano carbon signal is very low compared to that of the formyl carbon resonance in the starting formamides (4.16 a-d). The chemical shifts of alkyl and aryl substituents at the α- position did not vary significantly from their corresponding signals in the starting formamides (4.16 a-d).

In the $^{1}H$ n.m.r. spectra of isocyanides (4.17 a-d) (Table 4.6), the protons of the benzotriazole ring, and the protons of the various alkyl and aryl substituents, exhibited the expected ratios, chemical shifts, and multiplicities. The chemical shifts of the methylene and methine protons at the α-position of the isocyanide group did not change significantly from the starting formamides (4.16 a-d). The multiplicity pattern (Table 4.6) however is changed due to the absence of the NH proton which was (along

with the absence of any formyl proton resonances) indicative of product formation.

Table 4.6 $^1$H N.m.r. Chemical Shifts (δ) of N-(α-Benzotriazol-1-yl)alkyl Isocyanides (4.17 a-d).[a]

| Comp. No. | Bt | Bt-CH($R^1$) (m, J(Hz), H) | $R^1$ (m, J(Hz), H) |
|---|---|---|---|
| 4.17a | 7.3-8.3 (m, 4H) | 6.3 (s, 2) | - |
| 4.17b | 7.2-8.4 (m, 4H) | 6.5 (t, 8, 1) | 1.1 (t, 8, 3) |
| | | | 1.2-2.2 (m, 2) |
| | | | 2.5 (m, 2) |
| 4.17c | 7.2-8.4 (m, 4H) | 6.3 (d, 9, 1) | 0.8 (d, 6, 3) |
| | | | 1.4 (d, 6, 3) |
| | | | 2.4-3.2 (m, 1) |
| 4.17d | 7.2-8.4 (m, 4H) | 7.8 (s, 1) | 7.4 - 7.5 (m, 5) |

[a] In $CDCl_3$ with $Me_4Si$ as the reference.

### 4.3.3. Lithiation on Benzotriazolylmethyl Isocyanide (4.17a)

An attempted reaction between 1.1 equivalent of n-butyllithium and benzotriazolylmethyl isocyanide (4.17a) at -75°C in dry THF for 45 min, exhibited a color change from a yellow solution to dark brown suspension. Addition of $D_2O$ or benzophenone gave a dark brown solid which was attributed to displaced benzotriazole. Many repetitions under various conditions gave the same result. The results are not conclusive.

## 4.4. Summary and Conclusion

N-(Benzotriazol-1-yl)alkyl formamides (4.16 a-d) were synthesized in high yield via a one pot procedure which is also applicable to large scale preparations.

A general method for the synthesis of benzotriazolylalkyl isocyanides (4.17 a-d) from formamides (4.16 a-d) with good yields and high purity has also been developed. Isocyanides (4.17a) and (4.17d) are not malodorus.

## 4.5 Experimental

Melting points were determined on a Bristoline hot-stage microscope and are uncorrected. $^{1}H$ n.m.r. spectra were recorded on a Varian EM 360L, XL-200, or XL-300 spectrometer with tetramethylsilane as an internal standard. $^{13}C$ n.m.r. spectra were recorded on a Varian XL-200 (50 MHz), or XL-300 (75 MHz) spectrometer referring to the middle signal of $CDCl_3$ (77.0 ppm) or $Me_2SO$-$d_6$ (39.5 ppm). Infrared spectra were recorded on a Perkin Elmer 283B and FTIR spectrophotometer and only characteristic absorption bands are reported. Elemental analyses and HR/MS were performed under the supervision of Dr. D. H. Powel and Dr. R. W. King of the University of Florida Department of Chemistry.

4.5.1. General Procedure for Preparation of N-(α-Benzotriazol-1-yl)alkylformamides (4.16 a-d)

A mixture of benzotriazole (0.05 mole), the appropriate aldehyde (0.17 mol), formamide (0.5 mole) and toluene (20 mL) was refluxed with a Dean Stark trap for the appropriate length of time, and the water collected was removed. Two methods were adopted for the isolation of the product.

Method A: Sodium chloride (27% solution, 60 mL) was added to the reaction mixture which was kept in the refrigerator overnight. The product precipitated, was filtered, and washed with water and ether.

Method B: Ethyl acetate (30 mL) and water (30 mL) were added to the reaction mixture and the aqueous layer was separated. To the aqueous layer another 30mL of ethyl acetate was added. The combined organic layers were washed with saturated sodium carbonate solution (3x30 mL) and again with water (10 mL). After drying over magnesium sulfate the solvent was removed under reduced pressure to give a yellow oil.

4.5.1.1. Preparation of N-(benzotriazol-1-yl)methylformamide (4.16a) (Method A)

Benzotriazole, formamide and paraformaldehyde gave after 4 h, a solid (63%), (white needles from diethyl ether), m.p. 138-139 °C, lit., m.p. 146-147 °C [83MI723]. Anal. calcd. for $C_8H_8N_4O$: C, 54.54; H, 4.58; N, 31.80. Found C, 54.17; H, 4.42; N, 32.02. $\delta_H$ ($CDCl_3$/DMSO-$d_6$) 6.2

(2H, J = 7 Hz, d), 7.2-8.2 (4H, m), 8.2 (1H, s), 9.2-9.4 (1H, s). $\delta_C$ ($CDCl_3$/DMSO-$d_6$) 160.8, 144.6, 131.1, 126.4, 123.0, 118.1, 109.8, 48.2; $\nu_{max}$ ($CHBr_3$) 3230, 3030, 1680 $cm^{-1}$.

4.5.1.2. Preparation of N-(1-(benzotriazol-1-yl)butyl)-formamide (4.16b) (Method B)

Benzotriazole, formamide, and n-butyraldehyde gave after 12 h, a viscous yellow oil (87%). Calculated for $C_{11}H_{14}N_4O$ $M^+$, 218.11676; Found $M^+$ 218.11682; $\delta_H$ ($CDCl_3$) 0.9 (3H, J = 7 Hz, t), 1.1-1.8 (2H, m), 2.5 (2H, J = 7 Hz, q), 6.9 (1H, m), 7.2-8.4 (4H, m), 8.5 (1H, s), 9.3 (1H, J = 7 Hz, d); $\delta_C$ ($CDCl_3$) 161.9, 146.0, 133.3, 128.4, 125.0, 120.0, 110.9, 61.0, 36.3, 19.2, 13.8; $\nu_{max}$ ($CHBr_3$) 3250, 2960, 1675 $cm^{-1}$.

4.5.1.3. Preparation of N-(1-(benzotriazol-1-yl)-2-methyl)propylformamide (4.16c) (Method B)

Benzotriazole, formamide, and isobutyraldehyde gave after 12 h, a solid (92%), m.p. 93-95°C (white granules from diethyl ether). Anal. calcd. for $C_{14}H_{14}N_4O$: C, 60.59; H, 6.46; N, 25.67; Found C, 60.39, H, 6.02; N, 25.83. $\delta_H$ ($CDCl_3$) 0.7 (3H, J = 7 Hz, d), 1.2 (3H, J = 7 Hz, d), 2.2-3.2 (1H, m), 6.5 (1H, J = 9 Hz, t), 7.2-8.4 (5H, m), 8.6 (1H, s); $\delta_C$ ($CDCl_3$) 161.4, 145.2, 133.2, 127.9, 124.4, 119.5, 110.0, 65.9, 32.7, 18.8, 18.6; $\nu_{max}$ ($CHBr_3$) 3250, 2965, 1675 $cm^{-1}$.

4.5.1.4. Preparation of N-(1-(benzotriazol-1-yl)-1-phenyl)methylformamide (4.16d) (Method A)

Benzotriazole, formamide, and benzaldehyde gave after 12 h, a solid (77%), m.p. 141-142°C (white granules from ethanol). Anal. calcd. for $C_{14}H_{12}N_4O$: C, 66.65; H, 4.79; N, 22.20; Found C, 66.94; H, 4.82; N, 22.39; $\delta_H$ ($CDCl_3$/DMSO-$d_6$) 7.5 (5H, m), 7.6-8.2 (5H, m), 8.3 (1H, s), 10.2 (1H, J = 9 Hz, d); $\delta_C$ ($CDCl_3$/DMSO-$d_6$) 159.6, 144.2, 126.1, 118.0, 108.9, 122.6, 130.6, 124.9, 127.2, 124.4, 134.2, 61.8.

4.5.2. General Procedure for the Preparation of Isocyanides (4.17 a-d) from Formamides (4.16 a-d)

The corresponding formamide derivative (4.16) (0.1 mole) was dissolved in dichloromethane (100 mL) and then diisopropylamine (0.27 mole) was added. Phosphorus oxychloride (0.11 mole) was added in drops with stirring at 0°C. Stirring was continued for 1 h at 0°C, and in the case of the sparingly soluble formamide (4.16d) for 2 h at room temperature. A solution of sodium carbonate (20%, 100 mL) was added slowly. After stirring at room temperature for 1 h, dichloromethane (50 mL) and water (100 mL) were added. The organic layer was washed with water (3x50 mL) and dried over sodium sulfate. The solvent was evaporated under reduced pressure to give the isocyanides (4.17 a-d).

4.5.2.1. Preparation of N-(benzotriazol-1-yl)methyl isocyanide (4.17a)

Prepared (66%) from N-(benzotriazol-1-yl)methyl formamide (4.16a), di-isopropylamine, and phosphorus oxychloride after 2 h, as a solid (yellow crystals from benzene), m.p. 104-106°C, lit., m.p. 103-106°C [83MI723]. $\delta_H$ ($CDCl_3$) 7.3-8.3 (4H, m), 6.3 (2H, s). $\delta_C$ ($CDCl_3$) 163.3, 146.1, 131.7, 128.9, 124.9, 120.4, 108.8, 52.3; $\nu_{max}$ ($CHBr_3$) 2132 $cm^{-1}$.

4.5.2.2. Preparation of 1-(benzotriazol-1-yl)butyl isocyanide (4.17b)

Prepared (64%) from N-(1-(benzotriazol-1-yl)n-butyl)formamide (4.16b), di-isopropyl amine and phosphorus oxychloride after 1 h as a viscous yellow oil. Calculated for $C_{11}H_{12}N_4$ $M^+$, 200.10620; Found $M^+$, 200.10666. $\delta_H$ ($CDCl_3$) 1.1 (3H, J = 8 Hz, t), 1.2-2.2 (2H, m), 2.5 (2H, m), 6.5 (1H, J = 8 Hz, t), 7.2-8.4 (4H, m); $\delta_C$ ($CDCl_3$) 162.3, 147.3, 131.3, 128.4, 124.7, 120.4, 109.6, 66.3, 36.7, 18.0, 12.7; $\nu_{max}$ (neat) 2132 $cm^{-1}$.

4.5.2.3. Preparation of (1-(benzotriazol-1-yl)-2-methyl)propyl isocyanide (4.17c)

Prepared (72%) from N-(1-(benzotriazol-1-yl)2-methyl)propyl formamide (4.16c), di-isopropylamine, and phosphorus oxychloride after 2 h as a pale yellow oil which

on keeping upon storage at approximately at -20°C overnight gave a pale yellow solid m.p. 25-28°C. Calculated for $C_{11}H_{12}N_4$ $M^+$, 200.10620; Found $M^+$, 200.10663. $\delta_H$ ($CDCl_3$) 0.8 (3H, J = 6 Hz, d), 1.4 (3H, J = 6 Hz, d), 2.4-3.2 (1H, m), 6.3 (1H, J = 9 Hz, d), 7.2-8.6 (4H, m); $\delta_C$ ($CDCl_3$) 163.5, 146.7, 131.5, 128.9, 125.2, 120.9, 110.3, 72.9, 34.5, 19.0, 18.1; $\nu_{max}$ (neat) 2132 $cm^{-1}$.

4.5.2.4. Preparation of (1-(benzotriazol-1-yl)-1-phenyl)methyl isocyanide (4.17d)

Prepared (77%) from N-(1-benzotriazol-1-yl)-1-phenyl)methyl formamide (4.16d), di-isopropylamine, and phosphorus oxychloride after 2 h, as a viscous brown oil. Calculated for $C_{14}H_{10}N_4$ $M^+$, 234.09055; Found $M^+$ 234.09012; $\delta_H$ ($CDCl_3$) 8.0-8.1 (1H, m), 7.8 (1H, s), 7.3-7.4 (2H, m), 7.4-7.5 (5H, m), 7.1-7.2 (1H, m); $\delta_C$ ($CDCl_3$) 164.8, 147.1, 131.1, 130.8, 129.7, 128.9, 126.1, 124.8, 125.2, 120.9, 110.7, 69.3; $\nu_{max}$ ($CHBr_3$) 2132 $cm^{-1}$.

CHAPTER 5

# SYNTHESIS OF N,N'-DIALKYL- AND N,N,N'-TRIALKYL-FORMAMIDINES

## 5.1. Introduction

The chemistry of amidines has attracted much attention by chemists in connection with their wide scale employment in organic synthesis. The amidine group (5.1) is the nitrogenous analogue of carboxylic acids and esters. In general, an amidine is named after the acid or amide which may be obtained from it by hydrolysis; thus $HC(=NH)NH_2$ is formamidine. The amino and imino nitrogen atoms are referred to as N and N', respectively. Depending on the number and distribution of the substituents on N and N' nitrogen atoms, amidines may be classified into five types: unsubstituted, monosubstituted, N,N'-disubstituted, N,N-disubstituted and trisubstituted amidines.

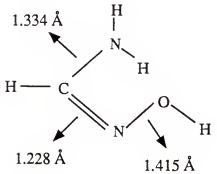
### 5.1.1. Structure of Amidines

The amidine group (5.1) combines the properties of an azomethine C=N double bond with an amide C-N single bond with partial double bond character as indicated by the mesomeric form (5.2).

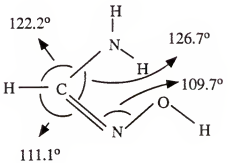
(5.1) (5.2)

Figure 5.1 Mesomeric Forms of Amidine

The nearest accurate structural data to an unsubstituted amidine in the crystalline state is formamidoxime (5.3) [65AC955].




(5.3)




(5.3)

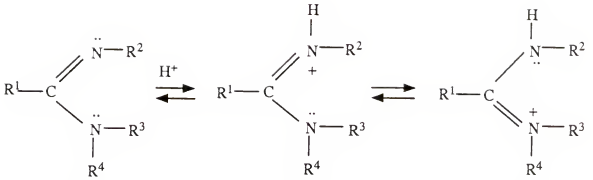
Figure 5.2 Structure of Formamidoxime

The skeleton of the formamidoxime molecule is completely planar showing a short C=N double bond (1.29 Å) which is only slightly longer than a pure unconjugated C=N double bond (1.27 Å). The C-N single bond distance (1.33 Å) is appreciably shorter than a pure single bond (1.47 Å) [70CB2902]. The planarity, the elongation of the double bond, and the shortening of the single bond reflect the amidine π-system resonance, indicated by mesomeric structures (5.1) and (5.2). The dipole moments of amidines lie in the range 2.2 to 3.4 D [75MI7].

### 5.1.2. Physical Properties of Amidines

#### 5.1.2.1. Basicity

Generally, amidines are stronger bases than aliphatic amines (eg. methylamine pKa 10.6; acetamidine pKa 12.4). Usually $sp^2$-hybridized imino nitrogen atoms are less basic than the $sp^3$ hybridized nitrogen of aliphatic amines due to its higher degree of s-character. One might therefore expect a decrease in basicity of amidines compared to amines as the protonation occurs at the imino nitrogen. The observed increase in basicity results from the complete delocalization of charge in the amidinium cation (Scheme 5.1) and hence its stabilization [61CA15128e, 73CA15210z].



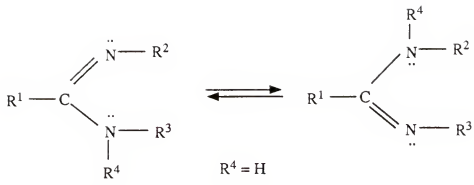
(5.1) (5.4) (5.5)

Scheme 5.1

In general, the basicity of the amidines depends to a greater extent on the substituents at the imino-nitrogen atom than on the substituents at the amino nitrogen atom [79IC254].

5.1.2.2. Tautomerization

The tautomerism of amidines has been extensively studied. Among the five types of amidines, only N-monosubstituted ($R^4$ = H) and N,N'-disubstituted amidines ($R^2 \neq R^3$) exhibit tautomerism in solution (Scheme 5.2). The two tautomeric forms are interconverted either directly, or by protonation and subsequent deprotonation [63AHC317].



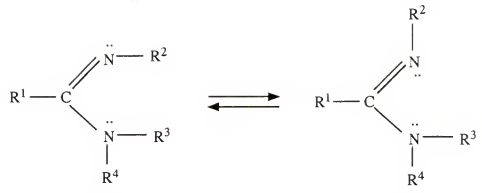

(5.1) (5.6)

Scheme 5.2

Infrared, $^{1}H$ n.m.r. [78CJC1470], and ultraviolet spectroscopy [76JCS(P2)211] are extremely useful tools for the study of tautomerism in amidines.

5.1.2.3. Cis and trans isomerization

The relative position of the substituents with respect to the C=N bond in amidines has attracted much attention. There is evidence to show that the Z isomer of formamidine is thermodynamically unstable and isomerizes to the E form [80TL885, 80CC130] (Scheme 5.3).

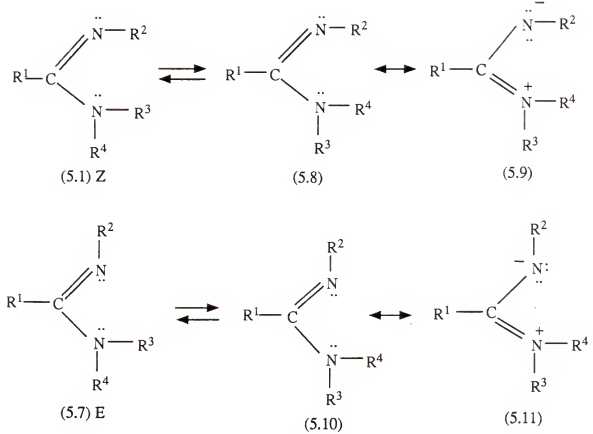


(5.1) Z (5.7) E

Scheme 5.3

5.1.2.4. Restricted rotation around the C-N single bond

A series of studies have been devoted to the hindered or restricted rotation around the C-N single bond in amidines. There is appreciable double bond character in the C-N single bond such that rotation around this bond is restricted. Consequently, the two N,N-substituents become magnetically non-equivalent (Scheme 5.4). Rotational barriers about the C-N bond have been determined by variable-temperature n.m.r. and they vary in the range 11-21 kcal $mol^{-1}$ [83RCR377].

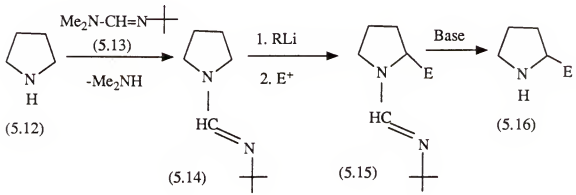


Scheme 5.4

5.1.3. Chemical Properties of Amidines

Although all amidines react with nucleophiles and electrophiles, more emphasis is given to the synthetic use of formamidines from this section onwards. The simplest reaction of amidines with nucleophiles is their hydrolysis. Usually hydrolysis occurs more rapidly in alkaline solution than in acidic solution. Substituents on both N and N'-

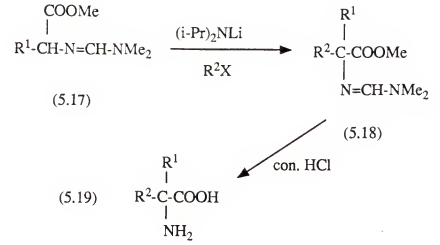
nitrogen atoms appear to influence the reactivity by both steric and inductive effects [75MI350]. An interesting route for the synthesis of substituted pyrazines was achieved by exploiting the conditions governing the retention and cleavage of the amidine group upon hydrolysis [80JOC2485]. The use of formamidines as protecting groups permitted the synthesis of an anthraquinone derivative, which is otherwise difficult to prepare [80ZOK1056]. Similarly, Meyers and co-workers have used formamidines as precursors to α-amino carbanions which are widely applied to asymmetric C-C bond forming reactions (Scheme 5.5) [85MI59].




Scheme 5.5

Another important reaction of amidines with nucleophiles, is the transamination reaction which is used as one of the stages in heterocyclic synthesis [78ACR314].

Among the reactions with electrophiles, much information is available on acylation [72CA61615, 80S133, 76MI1462, 77MI2157, 79CA156827] and fluorination [77MI2139, 76MI1963]. Amidines also react with alkyl halides [72JOC2960] and dichlorocarbene [75MI301]. A new method of α-substituted aminoacids synthesis based on the alkylation of N,N,N'-trisubstituted amidines has also been published recently (Scheme 5.6) [77JOC2639].




Scheme 5.6

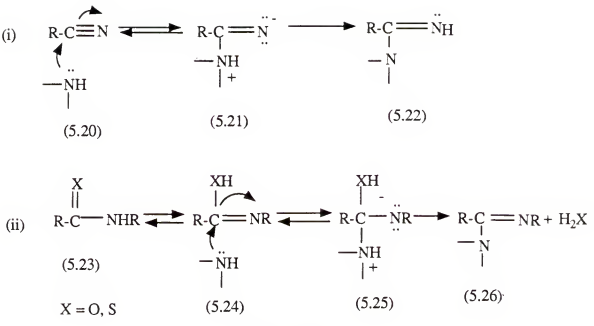
In these reactions of amidines with electrophiles, the processes involved either a nitrogen atom or a carbon atom of an alkyl group attached to a nitrogen atom. Based on their reactions with electrophiles and nucleophiles, amidines are widely used in the synthesis of various heterocycles [83RCR377]. Reversible intramolecular 1,3-migration of various groups within the amidine system is also well known [76ZOK1260, 76ZOK1271, 79ZOK1355, 80ZOK686].

### 5.1.4. Biological Properties of Amidines

Among the amidines, formamidines have the widest application in biological studies. They feature in the biochemical pathways associated with the anabolism of imidazoles and purines, and in the catabolism of histidine. Di- and tri-substituted formamidines, with aryl substituents at the imino nitrogen atom, are used as acaricides [74MI407], as antiulcer reagents acting as histamine $H_2$-receptor antagonists [84JMC380], and as herbicides [73CA136844j]. They are also useful in combatting bacterial, protozoal, viral, and helmintic pathogens [65CA11441c]. The trialkyl formamidine, 4-(diphenylmethyl)-1-[(imino)methyl]piperidine is proven to be a potent gastric antisecretary agent and anticholinergic agent [83JMC535].

### 5.1.5. Synthesis of Amidines

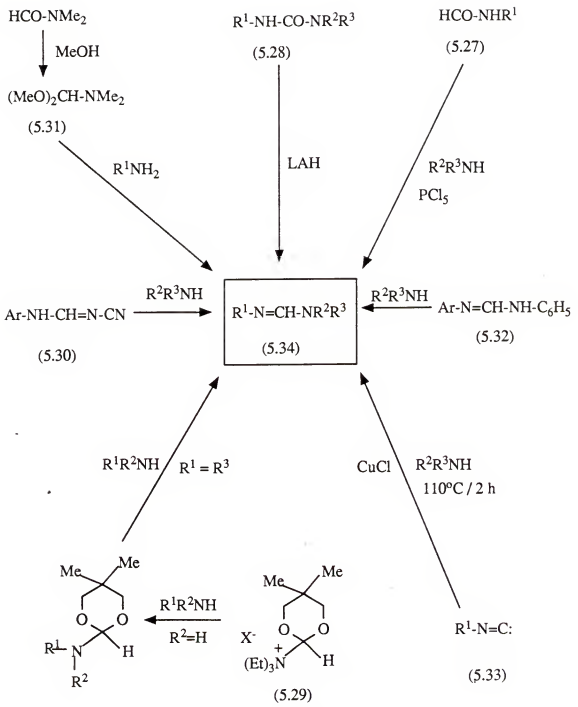
In general, amidines are synthesized from starting materials having unsaturated carbon nitrogen bonds; the introduction of the second nitrogen is realized by the action of ammonia, or a primary or secondary amine. The preparations of amidines (Scheme 5.7) can be divided into three groups based on the nature of the starting material: (i) from nitriles, (ii) from amides and thioamides, and (iii) from miscellaneous starting materials.



Scheme 5.7

Unfortunately, this general scheme holds good only for a few particular cases. The large variety of possible substituents on the carbon and nitrogen atoms complicates the classification. Methods of preparation are more, or less successful according to the nature of the substituents. Furthermore, in some cases, although based on the fundamental scheme described above, precursors or derivatives of the reactants are used.

The use of traditional nitrile starting materials [55JOC1569] has very limited application in formamidine synthesis ($R^1$ = H in 5.1). Formamidines are prepared from amides [54JAM3978, 85MI59], substituted urea [64JOC3967], dialkoxymethylammonium salts [73S423], N-cyanoformimidate [86S288], amidines [70RC453], and isocyanides [66TL6121] (Scheme 5.8).


HCO-NMe2
MeOH
(MeO)2CH-NMe2
(5.31)
R1NH2
R1-NH-CO-NR2R3
(5.28)
LAH
HCO-NHR1
(5.27)
R2R3NH
PCl5
Ar-NH-CH=N-CN
(5.30)
R2R3NH
R1-N=CH-NR2R3
(5.34)
R2R3NH
Ar-N=CH-NH-C6H5
(5.32)
R1R2NH
R1 = R3
CuCl
R2R3NH
110°C / 2 h
R1R2NH
R2=H
X-
(Et)3N
R1-N=C:
(5.33)
(5.29)

Scheme 5.8

Preparation of N,N'-di- (with dissimilar substituents at the N- and N'- positions) and N,N,N'-tri-substituted amidines is difficult. Clearly the use of a nitrile starting material is not possible, since it affords substitution only at the N-position. Most other methods yield similar substitutions at N and N' positions [44CR351]. Even among the existing amidines with dissimilar substituents, the aryl group is the most common substituent at the N' position with a methyl group at the N position. Hence, preparing formamidines with dissimilar substituents at N and N' positions becomes much more stringent for the reasons discussed above. Although N,N,N'-trialkyl-substituted formamidines are potent antiulcer agents [83JMC535], the literature on their synthesis is limited [64JOC3697, 66TL6121].

Among the existing methods, the method from isocyanide [66TL6121] is superior in yield, absence of side products, and generality of the method. This method usually involves one equivalent of isocyanide, three equivalent of an amine, and a catalytic amount of copper, silver or zinc salt. The reaction conditions require heating at 110°C for two hours. Similar attempts with the secondary amino group of tetrahydropterine [84HCA166] have failed. The reaction between cyclohexyl isocyanide and aromatic sulfonamide in the presence of copper chloride gave less than 10% of N-sulfonylformamidine, under refluxing conditions for more

than 25 days. If a compound also has another copper complexing group like benzotriazole [81JAM211] this method becomes even less successful. Although hypothetically any isocyanide can be used to obtain the target formamidine, the synthesis and malodor of low molecular weight isocyanides are major obstacles in isocyanide chemistry as discussed in chapter 4.

Hence, the development of odorless and highly reactive isocyanides should be valuable for the synthesis of a variety of formamidines, and an useful contribution to the field of medicine and agriculture.

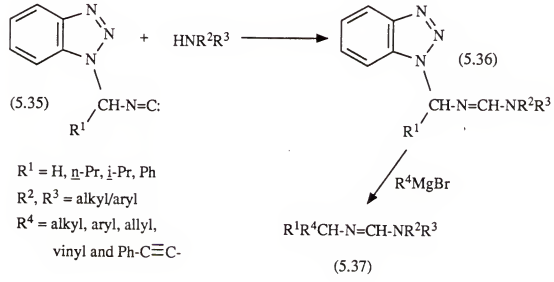
## 5.2. Objectives

A reactive amine hydrogen in a primary or secondary amine and the amine nitrogen can undergo 1,1-addition on an isocyano carbon to afford N,N'-disubstituted and N,N,N'-trisubstituted formamidines. The use of isocyanides with an α-activating group which is also a good leaving group would make the formamidine synthesis a more versatile one. Such strategy is novel although α-activated isocyanides are used for other synthetic purposes.

Benzotriazol-1-ylmethyl isocyanide (5.35a) is a odorless solid. The α-activating potential and the leaving group ability of benzotriazole are discussed in chapters 3 and 4.

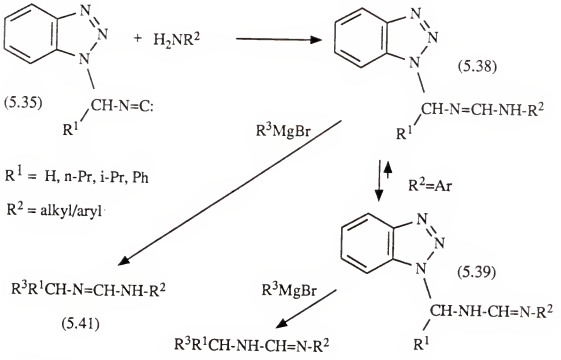
The goal of this work was to develop a general, versatile method for the preparation of N,N'-di- and N,N,N'-tri-substituted formamidines.

A 1,1-addition reaction of a secondary amine to α-(benzotriazol-1-yl)alkyl isocyanide (5.35) is potentially able to give trisubstituted formamidines (5.36) with desirable substituents at the N'-position. Displacement of the benzotriazole group by a variety of Grignard reagents makes it versatile by affording various possible combinations of trisubstituted formamidines (5.39) (Scheme 5.9). Such formamidines are generally difficult to obtain for reasons previously discussed (5.1.5).




Scheme 5.9

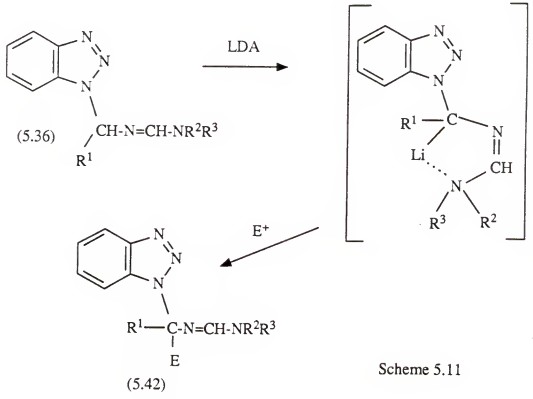
Similar insertion reactions of primary amines into α-(benzotriazol-1-yl)alkyl isocyanide (5.35), and subsequent displacement of the benzotriazole group by Grignard reagents, would give N,N'-disubstituted formamidines (5.40) and (5.41). With prudent choice of Grignard reagents, formamidines with dissimilar substituents at N and N' positions could be obtained (Scheme 5.10).




Scheme 5.10

Metalation and subsequent alkylation at the position α to the amine nitrogen atom has been well exploited by Myers et. al. [85MI59]. To date, a single reference on the

metalation and subsequent alkylation at the α-position to the imino nitrogen atom to give α-substituted aminoacids has been published [77JOC2639]. As discussed in chapter 4, the α-activating ability of benzotriazole can also be exploited in the formamidine system (5.36) to modify at the position α to the imino nitrogen atom also. Such a system may be used for further transformation to a desired goal, for example electrophilic substitution (5.42).




Scheme 5.11

## 5.3. Results and Discussion

### 5.3.1. Attempted Reaction of Benzotriazol-1-ylmethyl Isocyanide (5.35a) with Primary and Secondary Amines with Catalyst

A ternary mixture containing one equivalent of benzotriazol-1-ylmethyl isocyanide (5.35a), three

equivalents of a primary or secondary amine, and a catalytic amount (0.02 mmole) of copper iodide (zinc chloride for aryl amines) did not afford the expected formamidines under the conditions of heating at 110°C for two hours. Reactions were attempted with primary amines included n-butylamine, benzylamine, and aniline, and of secondary amines, piperidine and morpholine (5.35a). All presumably gave mainly a metallic complex. Benzotriazoles are known to form copper complexes [81JAM211].

### 5.3.2. Reaction of Benzotriazol-1-ylmethyl Isocyanide with Secondary Amines without Catalyst

The aforementioned result prompted an attempt to carry out the reaction of amines with isocyanide (5.35a) in the absence of a metal salt catalyst.

#### 5.3.2.1. Cyclic secondary amines (piperidine, pyrrolidine and morpholine)

Three equivalents of the secondary amine (piperidine, morpholine or pyrrolidine) with one equivalent of isocyanide (5.35a) at room temperature afforded practically pure N'-(benzotriazol-1-yl)methyl N,N-dialkylformamidines (5.36 a-c) within one hour in high yield (Table 5.1). Instead of three equivalents of amine, 1.1 equivalents of amine for a longer time gave quantitative conversion to the expected

formamidines (5.36 a-c). They were characterized by $^{1}H$ and $^{13}C$ n.m.r. (Tables 5.2 and 5.3), and infrared spectral data, and elemental analysis.

Table 5.1 Synthesis of N,N-Dialkyl-N'-(benzotriazol-1-yl)-alkylformamidines (5.36).

$$Bt\text{-}CH(R^1)\text{-}N{=}CH\text{-}NR^2R^3$$

(5.36) (a) = $R^1$ = H; $R^2R^3$ = $-(CH_2)_5-$

(b) = $R^1$ = H; $R^2R^3$ = $-(CH_2)_2-O-(CH_2)_2-$

(c) = $R^1$ = H; $R^2R^3$ = $-(CH_2)_4-$

(d) = $R^1$ = i-Pr $R^2R^3$ = $-(CH_2)_5-$

| Comp. No. | Yield (%) | Recryst. solvent | M.p. (°C) | Formula | Analysis (%) Required (Found) | | |
|---|---|---|---|---|---|---|---|
| | | | | | C | H | N |
| (5.36a) | 79 | $Et_2O$ | 99-102 | $C_{13}H_{17}N_5$ | 64.2 (64.3) | 7.0 (7.1) | 28.8 (29.2) |
| (5.36b) | 92 | $Et_2O$ | 116-119 | $C_{12}H_{15}N_5O$ | 58.8 (58.7) | 6.2 (6.2) | 28.6 (28.5) |
| (5.36c) | 89 | $Et_2O$ | 125-128 | $C_{12}H_{15}N_5$ | 62.9 (62.6) | 6.6 (6.6) | 30.5 (30.8) |
| (5.36d) | 99 | - | [a] | $C_{16}H_{23}N_5$ | 285.1949[b] (286.2034) | | |

[a] Crystallization attempts lead to rearrangement.

[b] HR/MS is reported.

Table 5.2 $^1$H N.m.r. Chemical Shifts (δ) of N,N-Dialkyl-N'-(benzotriazol-1-yl)alkylformamidines (5.36).[a]

$$Bt\text{-}CH(R^1)\text{-}N{=}CH\text{-}NR^2R^3$$

(5.36) (a) = $R^1$ = H; $R^2R^3$ = $-(CH_2)_5-$
(b) = $R^1$ = H; $R^2R^3$ = $-(CH_2)_2-O-(CH_2)_2-$
(c) = $R^1$ = H; $R^2R^3$ = $-(CH_2)_4-$
(d) = $R^1$ = i-Pr $R^2R^3$ = $-(CH_2)_5-$

| Comp. No. | Bt (m, 4H) | Bt-CHR$^1$ (s, 2H) | N=CH (s, 1H) | R$^2$NR$^3$ (m, H) |
|---|---|---|---|---|
| (5.36a) | 7.3-8.1 | 6.0 | 7.6 | 1.4-1.8 m 6, 3.0-3.6 bs 4 |
| (5.36b) | 7.3-8.2 | 6.1 | 7.7 | 3.2-3.5 m 4, 3.5-3.9 m 4 |
| (5.36c) | 7.3-8.2 | 6.0 | 7.9 | 1.7-2.0 m 4, 3.2-3.5 bs 4 |
| (5.36d)[b] | 7.0-8.2 | 5.5[c] | 8.1 | 1.0-1.8 m 6, 2.6-3.8 m 4 |

a In $CDCl_3$ with $Me_4Si$ as internal reference.

b i-Pr: 2.0-2.6 (m, 1H), 1.0 (bs, 3H), 0.8 (bs, 3H).

c Appears as a doublet (J = 7 Hz) for one hydrogen.

Table 5.3 $^{13}C$ N.m.r. Chemical Shifts (δ) of N,N-Dialkyl-N'-(benzotriazol-1-yl)alkylformamidines (5.36).[a]

$Bt\text{-}CH(R^1)\text{-}N{=}CH\text{-}NR^2R^3$

(5.36) (a) = $R^1$ = H; $R^2R^3$ = $-(CH_2)_5-$
(b) = $R^1$ = H; $R^2R^3$ = $-(CH_2)_2-O-(CH_2)_2-$
(c) = $R^1$ = H; $R^2R^3$ = $-(CH_2)_4-$
(d) = $R^1$ = i-Pr $R^2R^3$ = $-(CH_2)_5-$

| | Bt signals | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Comp. | C-3a | C-4 | C-5 | C-6 | C-7 | C-7a | Bt-$CH_2$ | N=CH |
| (5.36a)[b] | 132.6 | 123.6 | 110.4 | 119.5 | 126.9 | 146.1 | 68.1 | 156.8 |
| (5.36b)[c] | 133.8 | 124.4 | 110.8 | 120.3 | 127.8 | 147.0 | 68.1 | 157.0 |
| (5.36c)[d] | 133.1 | 123.5 | 110.2 | 119.3 | 126.8 | 146.0 | 67.7 | 154.6 |
| (5.36d)[e] | 132.0 | 123.0 | 113.0 | 119.3 | 126.2 | 147.0 | 88.2 | 155.6 |

a In $CDCl_3$ with $CDCl_3$ as internal reference.

b $R^2NR^3$ : 41-45 (b), 25.5, 24.4.

c $R^2NR^3$ : 43-45 (b), 67.9.

d $R^2NR^3$ : 44-50 (b), 24.6.

e $R^2NR^3$ : 45-52 (b), 24.5-28.0 (b), 24.6;
i-Pr : 18.5, 18.9 and 34.9.

The $^{13}C$ n.m.r. spectra (Table 5.3) of these compounds showed the characteristic pattern of 1-substituted benzotriazole. The carbon resonance for the methylene carbon attached to the benzotriazole group was observed in the range 67.8-68.0 ppm. The formyl carbon between the imino and amino nitrogen atoms falls in the range 154.7-157.2 ppm. An interesting contribution by dipolar resonance hybrids of trisubstituted amidines was detected from the n.m.r. data, which revealed the restricted rotation around the C-N single bond. At room temperature, the $\alpha$ and $\beta$ methylene carbons of the amino nitrogen atom showed broad signals between 40-52 ppm and 24-27 ppm, respectively. Variable temperature n.m.r. analysis in the range from room temperature to -50°C, displayed separate signals for $\alpha,\alpha',\beta,\beta'$ methylene carbons. Also as temperature is decreased there is an increase in the intensity of these four methylene carbons. Another important feature is that none of the low temperature n.m.r spectra showed another set of signals either for the benzotriazole ring carbons or for the methylene group between the benzotriazole group and the imino nitrogen. Similar results were obtained for all three adducts. One set of signals was observed for the substituent on the imino nitrogen at all temperatures studied and two sets of signals only for substituent groups on amino nitrogen. This interesting feature is not due to

cis trans isomerization around the C=N bond but only due to restricted rotation around the C-N single bond due to partial double bond character.

The $^{1}H$ n.m.r. spectra (Table 5.2) showed a reasonable pattern and range for 1-substituted benzotriazole. Methylene protons on carbon attached to benzotriazole were observed in the range 6.0-6.2 ppm and the formyl proton was observed in the usual range 7.7-7.8 ppm. As noted in $^{13}C$ n.m.r., $^{1}H$ n.m.r. also highlighted restricted rotation around the C-N single bond due to partial double bond character. At room temperature, the $\alpha$ and $\beta$ methylene protons appeared as broad singlets. As the temperature was lowered, the $\alpha,\alpha'$ methylene protons were observed as two broad separate singlets, however the $\beta$ methylene protons were still observed as broad singlet unlike the $\beta,\beta'$ methylene carbons of the same compound (Figures 5.3, 5.4 and 5.5).

Formamidines are known to exist in the thermodynamically more stable E form at room temperature [80TL885, 80JCS130]. The chemical shift of the formyl carbon [range 154.6-157.0 ppm] is in agreement to the reported E form of formamidines [75OMR326]. In addition, the (equivalent) methyl protons $\alpha$ to the amino nitrogen appear as a singlet in the Z form, over the temperature range 35 to -80°C [80JCS130]. Hence compounds (5.36 a-d) are likely to be in E form which is also close agreement with N-substituted formamidines [80OMR126].

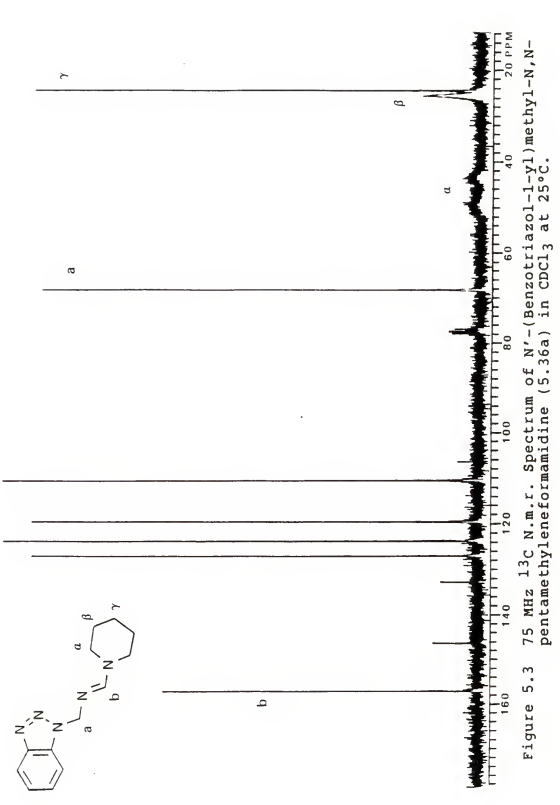



Figure 5.3 75 MHz $^{13}C$ N.m.r. Spectrum of N'-(Benzotriazol-1-yl)methyl-N,N-pentamethyleneformamidine (5.36a) in $CDCl_3$ at 25°C.

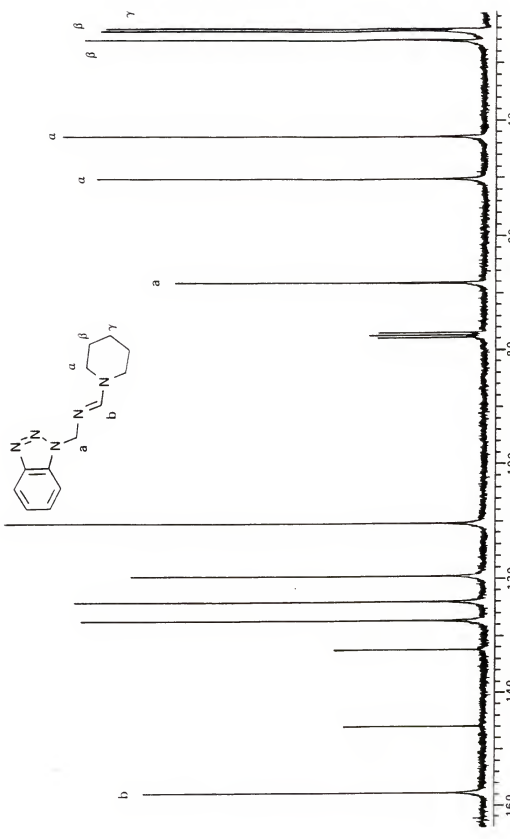



Figure 5.4 75 MHz $^{13}C$ N.m.r. Spectrum of N'-(Benzotriazol-1-yl)methyl-N,N-pentamethyleneformamidine (5.36a) in $CDCl_3$ at -50°C.

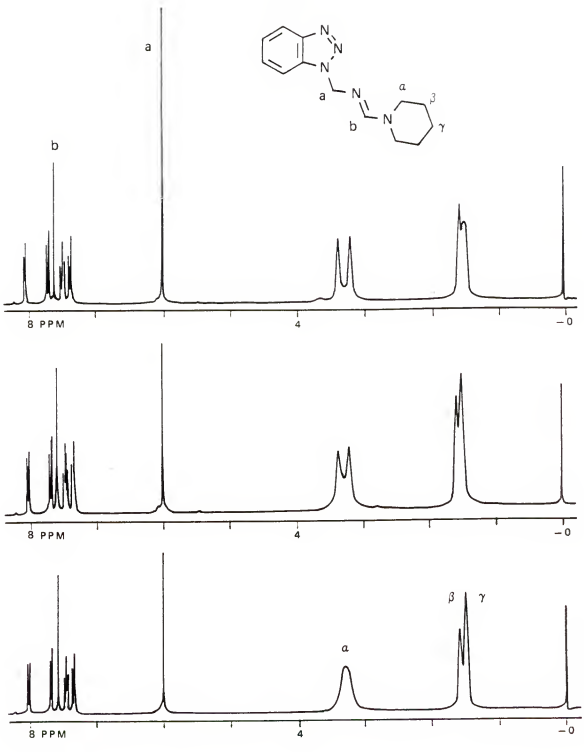



Figure 5.5 300 MHz $^1H$ N.m.r. Spectrum of N'-(Benzotriazol-1-yl)methyl-N,N-pentamethyleneformamidine (5.36a) in $CDCl_3$ at (a) 10°C, (b) 0°C and (c) -10°C.

Infrared spectral results show the characteristic C=N absorption peak at 1637 $cm^{-1}$. Variation in the substitution at the amino nitrogen did not affect the C=N absorption.

5.3.2.2. Synthesis of N,N,N'-trialkyl substituted formamidines by displacing benzotriazole from (5.36)

Reaction of one equivalent of N'-(benzotriazolylalkyl)-N,N-dialkylformamidines (5.36 a-d) in tetrahydrofuran with 1.1 equivalent of a Grignard reagent displaces the benzotriazole and affords the target title compounds in good yield (Table 5.4). The isolation procedure required several attempts to standardize the conditions, which might be due to the complexation of amidines with Grignard reagents [72BCSJ1846]. The structure of formamidines (5.37) were characterized by $^{13}C$ and $^{1}H$ n.m.r., and infrared spectral data and further supported by high resolution mass spectra. The displaced benzotriazole was also identified by thin layer chromatography and $^{1}H$ and $^{13}C$ n.m.r. spectra.

Table 5.4 Synthesis of N,N-N'-Trialkylformamidines (5.37)

$$R^1R^4CH\text{-}N{=}CH\text{-}NR^2R^3$$

(5.37) (a) = $R^1$ = H; $R^2R^3$ = $-(CH_2)_5-$ $R^4$ = Ph
(b) = $R^1$ = H; $R^2R^3$ = $-(CH_2)-O-(CH_2)-$ $R^4$ = Ph
(c) = $R^1$ = H; $R^2R^3$ = $-(CH_2)_4-$ $R^4$ = Ph
(d) = $R^1$ = H; $R^2R^3$ = $-(CH_2)_5-$ $R^4$ = p-Tolyl
(e) = $R^1$ = H; $R^2R^3$ = $-(CH_2)_5-$ $R^4$ = $CH_2{=}CH-$
(f) = $R^1$ = i-Pr; $R^2R^3$ = $-(CH_2)_5-$ $R^4$ = Ph

| Comp. No. | Yield | Formula | HR/MS Required | HR/MS Found |
|---|---|---|---|---|
| (5.37a) | 80 | $C_{13}H_8N_2$ | [a] | [a] |
| (5.37b) | 82 | $C_{12}H_{16}N_2O$ | 204.12625 | 204.12525 |
| (5.37c) | 79 | $C_{12}H_{16}N_2$ | 188.13135 | 188.13183 |
| (5.37d) | 62 | $C_{14}H_{20}N_2$ | 216.16265 | 216.16262 |
| (5.37e) | 76 | $C_9H_{16}N_2$ | 152.13135 | 152.13012 |
| (5.37f) | 53[b] | $C_{16}H_{24}N_2$ | [c] | [c] |

[a] Picrate, m.p. 136-137°C, lit. m.p. 139°C [84HCA166].

[b] Overall yield.

[c] Hydrochloride, m.p. 230-232°C.

Table 5.5 $^1$H N.m.r. Chemical Shifts (δ) of N,N,N'-Trialkylformamidines (5.37).[a]

| Comp. | $R^2NR^3$ (m, H) | $R^4$-CH-$R^1$ (m, H) | N=CH (m, 1H) | $R^4$ (m, H) |
|---|---|---|---|---|
| (5.37a) | 1.4-1.8 bs 6<br>3.2-3.6 bs 4 | 4.5 s 2 | 7.6 s | 7.2-7.6 (bs, 5H) |
| (5.37b) | 3.2-3.4 m 4<br>3.5-3.6 m 4 | 4.4 s 2 | 7.3 s | 7.1-7.3 (bs, 5H) |
| (5.37c) | 1.8-1.9 m 4<br>3.4-3.5 m 4 | 4.4 s 2 | 7.7 s | 7.0-7.5 (m, 5H) |
| (5.37d) | 1.6-1.8 bs 6<br>3.4-3.6 bs 4 | 4.5 s 2 | 7.7 s | 2.3 (s, 3H),<br>7.1-7.2 (m, 2H),<br>7.2-7.4 (m, 2H) |
| (5.37e) | 1.3-1.8 bs 6<br>3.4-3.7 bs 4 | 4.0 s 2 | 7.8 s | 5.9-6.1 (m, 1H),<br>5.1-5.3 (m, 2H) |
| (5.37f)[b] | 1.6-1.8 bs 6<br>3.4-3.7 m 2<br>4.1-4.4 m 2 | 4.2 t 1 | 8.3 s | 7.2-7.5 (m, 3H),<br>7.6-7.8 (m, 2H) |

[a] In $CDCl_3$ with $Me_4Si$ as internal reference, see Table 5.4 for the different R groups.

[b] $R^1$ = i-propyl group: 0.8 (d, J = 7Hz, 3H), 1.2 (d, J = 7Hz, 3H), 2.7-2.9 (m, 1H); NH = 4.2 (bs, 1H).

Table 5.6 $^{13}C$ N.m.r. Chemical Shifts (δ) of N,N,N'-Trialkylformamidines (5.37)[a].

| Comp. | $R^4$-CH | N=CH | $R^4$ | $R^1$ |
|---|---|---|---|---|
| (5.37a)[b] | 59.1 | 155.7 | 128.6, 127.9, 126.8, 142.1 | - |
| (5.37b)[c] | 59.4 | 154.8 | 128.1, 127.3, 126.3, 141.5 | - |
| (5.37c)[d] | 57.8 | 152.8 | 128.2, 127.6, 126.5, 141.1 | - |
| (5.37d)[e] | 54.9 | 154.5 | 129.2, 127.8, 129.0, 136.3 | - |
| (5.37e)[f] | 52.9 | 154.2 | 116.6, 135.3 | - |
| (5.37f)[g] | 71.9 | 153.6 | 128.3, 129.1, 129.7, 140.8 | 20.3 |
| | | | | 21.1 |
| | | | | 32.7 |

[a] In $CDCl_3$ with $Me_4Si$ as internal reference, see Table 5.4 for the different R groups.

[b] $R^2NR^3$: 47.3, 26.0, 25.1.

[c] $R^2NR^3$: 45.8, 66.5.

[d] $R^2NR^3$: 47.4, 24.9.

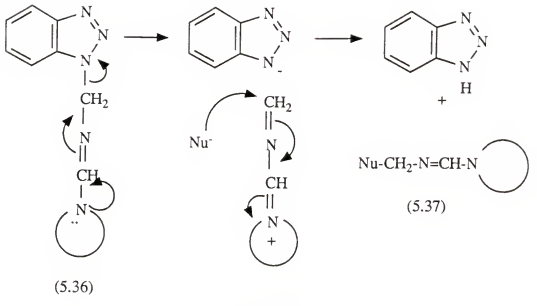
[e] $R^2NR^3$: 48.1, 25.4, 20.1.

[f] $R^2NR^3$: 45.1, 25.3, 23.7.

[g] $R^2NR^3$: 53.8, 48.2, 26.7, 25.3, 23.6, 32.7, 21.1, 20.3; Isolated as hydrochloride salt: hence α,α',β,β'-carbons display separate signals at 25°C.

The substituent alkyl or aryl groups, introduced by Grignard reagents, displayed reasonable chemical shifts for protons and carbons (Tables 5.5 and 5.6). The resonances of the formyl carbons of (5.37) are shielded about 1-2 ppm from their corresponding starting formamidines (5.36), with benzotriazole α to the imino nitrogen. This could be attributed to the electronegativity of the benzotriazole group in the starting formamidine (5.36). The same logic supports the added shielding of the methylene carbon signals α to the imino nitrogen atom. This additional shielding is about 9-10 ppm. All the N,N-dialkyl substituents showed the expected chemical shifts. All formamidines (5.37) also displayed the hindered rotation phenomenon as discussed in sections 5.1.2.4 and 5.3.2.1. There is little change in this regard due to the alkyl or aryl substitution in place of benzotriazole. At room temperature, the carbon signal α to the amino nitrogen atom appears as a singlet instead of total broadening as in (5.36). Replacement of benzotriazole by alkyl or aryl group did not affect the chemical shifts of either the formyl proton or the methylene protons on the amino nitrogen atom. But the methylene protons α-to the imino nitrogen were shielded about 1.5-2.0 ppm.

The displacement of benzotriazole could have occurred in two step pathway rather than simple $SN_2$ displacement (Scheme 5.12). This prediction is further substantiated from additional experimental evidence obtained and discussed in forthcoming sections.




(5.36)

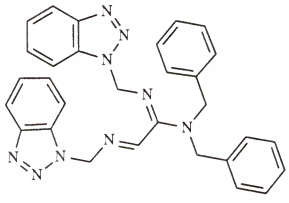
(5.37)

Scheme 5.12

5.3.2.3. Acyclic secondary amine (dibenzylamine)

An equimolar ratio of isocyanide (5.35a) and the acyclic secondary amine, dibenzylamine, afforded a bis product (5.43) in 68% yield. Similar reaction products are reported in the literature for the reaction of phenyl isocyanide with phenol and naphthols [71MI75]. The compound (5.43) was characterized on the basis of $^{13}C$ and $^{1}H$ n.m.r. spectral data and elemental analysis. $^{13}C$ n.m.r. displayed two sets of signals for the two moieties of 1-substituted benzotriazole as well as for the methylene carbons attached to benzotriazole. Their chemical shifts are different from each other as well as from the starting isocyanide (5.35a).

The formyl carbon resonance at 155.6 ppm and formyl proton resonance at 7.5 ppm indicate the presence of the iminoformyl group. Carbon and proton signals also account for dibenzylamine linked in the amidine group.

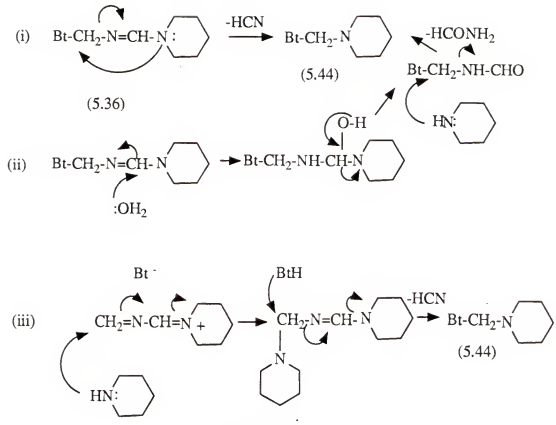


(5.43)

The same reaction with three equivalent of amine resulted in (benzotriazol-1-ylmethyl)dibenzylamine.

5.3.2.4. Rearrangement in N'-(benzotriazol-1-yl)methyl-N,N-dialkylformamidine

During the investigation of the reaction between (benzotriazol-1-yl)methyl isocyanide (5.35a) and piperidine in the molar ratio 1:3, an unexpected result was observed. After isolation of the pure expected formamidine (5.36), a rearranged product was isolated from the mother liquor after long standing at room temperature. The melting point, elemental analysis, $^{13}C$ and $^{1}H$ n.m.r., and infrared spectral data agreed to the literature reported values for 1-(benzotriazol-1-ylmethyl)piperidine [84HCA166].




Scheme 5.13

The rearrangement could occur by many possible mechanistic pathways (Scheme 5.13): (i) an intramolecular migration of the amine with the expulsion of hydrogen cyanide, (ii) water catalyzed migration of the amine with the expulsion of formamide, (iii) amine catalyzed rearrangement with the expulsion of hydrogen cyanide and amine. In order to explore the possible mechanism for the rearrangement, attempts were made on compound (5.36), by refluxing (5.36) in (i) dry benzene or toluene, (ii) benzene with equivalent amount of the same secondary amine, (iii)

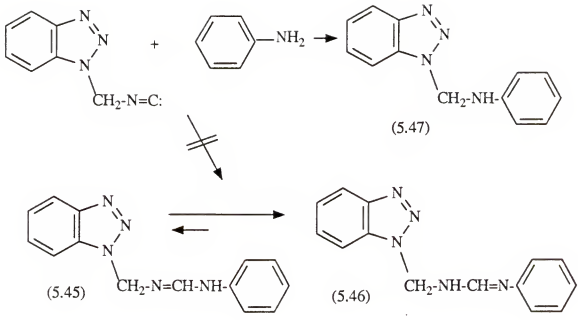
benzene with different secondary amine, (iv) benzene with a catalytic amount of same secondary amine and (v) benzene with water. All reactions were followed by thin layer chromatography, $^{1}H$ and $^{13}C$ n.m.r. and infrared spectra.

The pure formamidine (5.36) refluxed in dry benzene or toluene for 24 hours showed no rearrangement. Water was also found not to be the cause for rearrangement. This excludes the possibilities of (i) and (ii) in Scheme 5.13. In contrast with added amine (the same secondary amine or a different secondary amine) either in an equivalent amount or in a catalytic amount, the rearrangement product resulted (5.44). This implies that excess piperidine in the mother liquor from the preparation of formamidine (5.36a), catalyzes the rearrangement to give piperidinomethyl-benzotriazole (5.44). Hence the more likely mechanism for this rearrangement is via (iii) in Scheme 5.13.

### 5.3.3. Reaction of Benzotriazol-1-ylmethyl Isocyanide (5.35a) with Primary Amines

#### 5.3.3.1. Aromatic amines

Either one equivalent or three equivalents of aniline with one equivalent of benzotriazol-1-ylmethyl isocyanide at



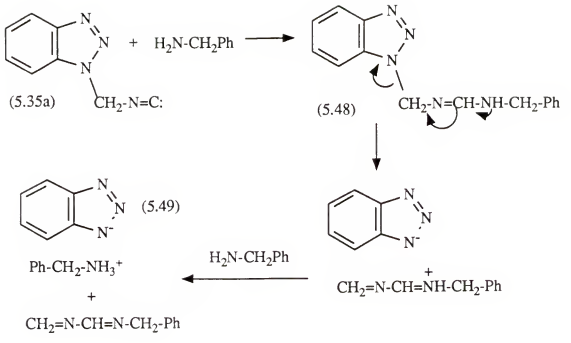

Scheme 5.14

room temperature gave a solid. N,N'-Disubstituted formamidines should show tautomerism and hence the most stable tautomer (5.46) is expected to form (Scheme 5.14). $^{13}C$ and $^{1}H$ n.m.r. spectral data account for all carbons and protons except for the formyl carbon and proton. The methylene protons on the carbon attached to benzotriazole are observed as a doublet due to NH coupling and this coupling collapsed to a singlet when the NH was exchanged with deuterium. In addition, a singlet was observed for the aforementioned methylene protons in the proton decoupled n.m.r. spectrum. The existence of two tautomers should be identified in an infrared spectrum as two peaks for the C=N

in the range 1630 $cm^{-1}$ and 1500 $cm^{-1}$, respectively. Two peaks were indeed observed at 1613 $cm^{-1}$ and 1495 $cm^{-1}$. In the infrared spectrum there was no N=C absorption peak (2131 $cm^{-1}$) of the starting isocyanide (5.35a). With these results it was suspected that, perhaps due to tautomerism, the formyl carbon and proton are not observed at room temperature. Variable temperature n.m.r. from 35 to -50°C showed no change in either in $^{13}C$ or in $^{1}H$ resonances. The confirmed structure of the rearranged product (5.44) lead the possibility, that it could be N-(benzotriazol-1-ylmethyl)aniline (5.47). In this case there is no formyl carbon or proton. The elemental analysis supported this conjecture and the melting point agreed to the literature value. A likely mechanism could be similar as in Scheme 5.13.

5.3.3.2. Aliphatic amines

Reaction of three equivalents of benzylamine with isocyanide (5.35a) afforded a salt, benzylammonium benzotriazolate (5.49). $^{13}C$ and $^{1}H$ n.m.r. spectral data provided structural evidence for (5.49) which was further confirmed by elemental analysis. Formation of the salt (5.49) could possibly be as outlined in Scheme 5.15.




Scheme 5.15

Reaction between n-butylamine (1.1 equivalent) and (5.35a) also gave a similar result as with benzylamine, producing the n-butylammonium benzotriazolate salt which was identified from its $^{1}H$ and $^{13}C$ n.m.r. spectra.

The possibility that the primary amine produced salts due to lowered steric hindrance, lead to the choice of neopentylamine and 1-methyl-n-butylamine. These amines also produced a similar result as with benzylamine. Hence the steric factor is not controlling this phenomenon of salt formation. As shown in scheme 5.15, the newly formed formamidine (5.48) becomes unstable due to the electron delocalization from the amino nitrogen and consequently expels the benzotriazole group.

Hence, subsequent attempts on other α-(benzotriazolyl)alkyl isocyanides were made with secondary amines.

### 5.3.4. Reaction of α-(Benzotriazol-1-yl)alkyl Isocyanides (5.35 b-d) with Secondary Amines

Table 5.7 shows the repeated attempts to synthesize N,N,N'-trisubstituted formamidines from isocyanides (5.35 b-d). A similarity (observed in all cases), is that the use of more than 1.1 equivalent of amine results either in rearranged product or the salt of the secondary amine and benzotriazole. With isocyanides (5.35 b and c) 1.1 equivalent of amine at room temperature for a longer duration of about three days to one week yielded the expected product which was characterized by $^{13}C$ and $^{1}H$ n.m.r., infrared, and high resolution mass spectroscopy. Attempted isolation of the product led to the formation of a rearranged product. Consequently the crude product was used for further transformation. With isocyanides (5.35d) none of the attempts became successful.

Table 5.7 Reaction of α-(Benzotriazol-1-yl)alkyl Isocyanides (5.35 b-d) with Secondary Amines

| Comp. No. | -N=C: No. of equiv. | Amine Name | Amine No. of equiv. | Solvent[a] | Time | Result[b] |
|---|---|---|---|---|---|---|
| 5.35b | 1 | Piperidine | 3.0 | - | 30 min. | SA |
| | 1 | Piperidine | 1.5 | DE | 30 h. | SA |
| | 1 | Piperidine | 1.1 | DE | 1 wk. | RA |
| 5.35c | 1 | Piperidine | 3.0 | - | 30 min | SA |
| | 1 | Piperidine | 3.0 | DE | 12 h. | SA |
| | 1 | Piperidine | 1.5 | - | 48 h. | SA |
| | 1 | Piperidine | 1.0 | - | 1 wk. | SA |
| | 1 | Piperidine | 1.0 | $Et_2O$ | 48 h. | RA |
| | 1 | Piperidine | 1.1 | DE | 76 h. | 5.36d |
| | 1 | Morpholine | 1.1 | DE | 3 wk. | I |
| 5.35d | 1 | Piperidine | 3.0 | $CH_2Cl_2$ | 28 h. | SA |
| | 1 | Piperidine | 1.3 | - | 3 wk. | SA |
| | 1 | Piperidine | 1.2 | $CH_2Cl_2$ | 22 h. | RA |
| | 1 | Piperidine | 1.1 | - | 46 h. | SA |
| | 1 | Piperidine | 1.1 | $CH_2Cl_2$ | 29 h. | SA |
| | 1 | Di-i-Pr-amine | 1.1 | $CH_2Cl_2$ | 2 wk. | SA |

[a] DE = Di-isopropylether.

[b] SA = quarternary salt of amine and benzotriazole, RA = rearranged product (aminoalkylbenzotriazole), and I = incomplete.

Displacement of benzotriazole on (5.36d) by Grignard reagent resulted in N,N,N'-trialkyl substituted formamidines with overall yield 53%. Its structure was characterized by $^{13}C$ and $^{1}H$ n.m.r. (Tables 5.5 and 5.6), infrared, and high resolution mass spectra as the corresponding hydrochloride salt. The chemical shifts for carbon and proton signals were reasonable. As expected the hydrochloride salt showed restricted rotation, hence $\alpha,\alpha',\beta,\beta'$ carbon signals as well as methylene protons attached on them are separately seen, even at room temperature (Table 5.5).

## 5.4. Conclusions

The strategy of using an electron withdrawing group substituted at the α-position of an alkyl isocyanide for the synthesis of N,N'-disubstituted and/or N,N,N'-trisubstituted formamidines is novel, although such isocyanides are known (eg. TOSMIC) in the literature [77JOC1153, 77JOC3114, 82MI4119] and have been used for other synthetic purposes. The combination of the electron withdrawing nature and leaving group ability of the benzotriazole group at the α-position of isocyanide makes the formamidine synthes is a versatile method (Schemes 5.9 and 5.10).

N,N-Cycloalkyl-N'-alkylformamidines with dissimilar substituents at N and N' positions have been prepared in

good yield. The method can be successfully carried out on a 0.1 mole scale. On comparing the reaction conditions, for the reaction of secondary amines with isocyanide (5.35a), and the existing analogous method [66TL6121], this method certainly has many advantages of : (i) the reacting amine does not have to be necessarily in three equivalent excess, (ii) catalysts (eg. copper salts) are not needed and alkyl isocyanides generally do not react with amines without catalysts even at higher temperature, (iii) the reaction is carried out at room temperature which is milder than in the literature method (110°C), (iv) duration of the reaction varies from five minutes to one hour depending upon the amine used, (v) isolation procedure of the product (5.36) is very simple and the crude product obtained often gave good elemental analysis, and (vi) another very important aspect of this method is that the starting isocyanide (5.35a) is a solid and non-malodorous.

This method will be highly valuable in the field of medicinal chemistry, since trialkyl formamidines (eg. 4-(diphenylmethyl)-1-[(imino)methyl]piperidine and its analogues) are potent oral gastric antisecretary agents and anticholinergic agents. This agent is currently prepared by a three or four step method in poor yield [83JMC535].

Comparing the reactivity of the α-(benzotriazol-1-yl)alkyl isocyanides (5.35 a-d) studied towards amines, the substituents at the α-position follow the order H > alkyl > aryl. A comparison of primary and secondary amines towards (5.35a) could not be made since the product identified from primary amines (5.45) and (5.48) is very unstable compared to the products obtained from secondary amines (5.36). The aforementioned problem with primary amines could be circumvented by using a protected primary amine like N-trimethylsilyl primary amine or by using an amide like aryl sulfonamide.

Generally the isocyanides (5.35 a-d) with an excess of amine and prolonged reaction duration leads to undesirable rearrangement, affording 1-(aminoalkyl)benzotriazoles, which are easily obtained by another method [87JCS(P1)781]. The use of more than 1.1 equivalent of amine, in the reaction with alkyl or aryl substituents at the α-position (5.35 b-d), leads to undesired products. Expected products may be achieved via Scheme 5.11.

## 5.5. Experimental

Melting points were determined on a Bristoline hot-stage microscope and are uncorrected. $^{1}H$ n.m.r. spectra were recorded on a Varian EM 360L, XL-200, or XL-300

spectrometer with tetramethylsilane as an internal standard. $^{13}C$ n.m.r. spectra were recorded on a Varian XL-200 (50 MHz), or XL-300 (75 MHz) spectrometer referring to the middle signal of $CDCl_3$ (77.0 ppm) or $Me_2SO$-$d_6$ (39.5 ppm). Infrared spectra were recorded on a Perkin Elmer 283B and FTIR spectrophotometer and only characteristic absorption bands are reported. Elemental analyses and HR/MS were performed under the supervision of Dr. D. H. Powel and Dr. R. W. King of the University of Florida Department of Chemistry.

5.5.1. General Procedure for the Preparation of N'-(α-Benzotraizol-1-yl)alkyl-N,N-dialkylformamidines (5.36 a-d) and Rearranged Products (5.43, 5.44, 5.47 and 5.49)

Method A

Benzotriazol-1-ylmethyl isocyanide (5.35a) (0.03 mol) was added in small portions to the stirring amine (0.1 mol) at room temperature. Reaction was exothermic as addition of isocyanide proceeded. After the completion of addition the reaction mixture was continued stirring at room temperature. Depending upon the amine used white solid was formed with 5 min to 1 hour. Formed white solid hindered the stirring. Then added diethyl ether (30 mL) and filtered the solid. The solid gave good elemental analysis without recrystallization.

Method B

Benzotriazol-1-ylalkyl isocyanide (5.35 a-d) (0.005 mole) of isocyanide was added in small portions to the stirring amine (0.0055 mol) in methylene chloride (5 mL) or diisopropyl ether (5 mL) at room temperature. Reaction was followed by infrared spectroscopy until the disappearance of isocyanide N=C absorption peak, 2131 $cm^{-1}$. Excess amine and the solvent were removed under reduced pressure. The residue was recrystallized in (5.36 a-c) and it was characterized and used without recrystallization in (5.36d).

5.5.1.1. Preparation of N'-(benzotriazol-1-yl)methyl-N,N-pentamethyleneformamidines (5.36a)

Prepared from benzotriazol-1-ylmethyl isocyanide and piperidine. Method A: 30 min, yield 79%; Method B: 12 h, yield 100%; (white needles from diethyl ether) m.p. 99-102°C. Anal. calcd. for $C_{13}H_{17}N_5$ : C, 64.17; H, 7.04; N, 28.78; Found C, 64.25; H, 7.13; N, 29.21. $^1H$ and $^{13}C$ n.m.r. data are in Table 5.2 and 5.3. $\nu_{max}$ ($CHBr_3$) 1637 $cm^{-1}$.

5.5.1.2. Preparation of N'-(Benzotriazol-1-yl)methyl-N,N-(3-oxapentamethylene)formamidine (5.36b)

Prepared from benzotriazol-1-ylmethyl isocyanide and morpholine. Method A: 1 h, yield 92%; Method B: 24 h, yield %; (white flakes from diethyl ether) m.p. 116-119°C. Anal. calcd. for $C_{12}H_{15}N_5O$ : C, 58.76; H, 6.16; N, 28.55; Found C, 58.67; H, 6.15; N, 28.51.

5.5.1.3. Preparation of N'-(Benzotriazol-1-yl)methyl-N,N-tetramethyleneformamidine (5.36c)

Prepared from benzotriazol-1-ylmethyl isocyanide and pyrrolidine. Method A: 5 min, yield 89%; Method B: h, yield 100%; (colorless flakes from diethyl ether) m.p. 125-128°C. Anal. calcd. for $C_{12}H_{15}N_5$ : C, 62.86; H, 6.59; N, 30.54. Found C, 62.58; H, 6.62; N, 30.76; $\nu_{max}$ ($CHBr_3$) 1637 $cm^{-1}$.

5.5.1.4. Preparation of N'-[1-(benzotriazol-1yl)-2-methyl]propyl-N,N-pentamethyleneformamidine (5.36d)

Prepared from 1-(benzotriazol-1-yl)methyl-2-methyl-propyl isocyanide (5.35c) and piperidine. Method B; yield 99%. The crude product was characterized. Attempts on crystallization lead to rearranged aminoalkylbenzotriazole. $^1H$ and $^{13}C$ n.m.r. data are given in Tables 5.2 and 5.3, respectively. Calculated for $C_{16}H_{23}N_5$, $M^+$ 285.1949; Found $M^+$ 286.2034; $\nu_{max}$ ($CHBr_3$) 1637 $cm^{-1}$.

5.5.1.5. Rearranged product N-(benzotriazol-1-yl)methylaniline (5.47) from (5.35a) and aniline

Prepared from benzotriazol-1-ylmethyl isocyanide (5.35a) and aniline as solid. Method A: 12 h, yield 67%; Method B: 2 wk. yield 82%; m.p. 139°C, lit. m.p. 138-139°C

[55JAM5386] (white needles from methanol). Anal. calcd. for $C_{13}H_{12}N_4$, C, 69.65; H, 5.39; N, 24.99. Found C, 69.62; H, 5.37; N, 25.15. $^{13}C$ N.m.r. δ ($CDCl_3$) 57.6, 109.1, 113.2, 119.2, 119.4, 123.4, 126.8, 128.8, 131.5, 144.0, 146.9. $^1H$ N.m.r. δ ($CDCl_3$) 5.1 (t, 1H, J 7Hz), 6.1 (d, 2H, J 7Hz), 6.7-8.1 (m, 9H); $\nu_{max}$ ($CHBr_3$) 1600 and 1510 $cm^{-1}$.

5.5.1.6. Rearranged product, N-(benzotriazol-1-yl)methyl-dibenzylamine from (5.35a) and dibenzylamine

Prepared from benzotriazol-1-ylmethyl isocyanide (5.35a) and dibenzylamine as solid. Method A: 2 h, yield 69% (white needles from methanol), m.p. 121-122°C, lit. m.p. 121-123°C [46JAM2496]. Anal. calcd. for $C_{21}H_{20}N_4$ : C, 76.80; H, 6.14; N, 17.06; Found C, 76.54; H, 6.16; N, 16.96. $^{13}C$ δ ($CDCl_3$) 55.1, 63.5, 109.2, 118.9, 123.0, 126.4, 126.7, 127.7, 128.2, 133.2, 137.2, 143.2. $^1H$ δ ($CDCl_3$) 3.8 (s, 4H), 5.5 (s, 2H), 7.1-8.5 (m, 14H).

5.5.1.7. Formation of benzylammonium benzotriazolate (5.49) from (5.35a) and benzylamine

Prepared from benzotriazol-1-ylmethyl isocyanide (5.35a) and benzylamine as solid. Method A: 12 h, yield 73%; m.p. 95-97°C; (white flakes from diethyl ether). Anal. calcd. for $C_{13}H_{14}N_4$ : C, 69.00; H, 6.24; N, 24.76; Found C, 69.24; H, 6.29; N, 24.72; $^{13}C$ δ ($CDCl_3$) 45.3, 114.4, 124.7, 126.5, 126.6, 128.0, 132.0, 138.7, 141.2; $^1H$ δ ($CDCl_3$) 4.1 (s, 2H), 6.4 (s, 3H); 7.3-7.7 (m, 7H), 7.9-8.3 (m, 2H).

5.5.1.8. Preparation of α-(benzotriazol-1-ylmethyl)-N'-(benzotriazol-1-ylmethyl)-N,N-dibenzylacetamidine (5.43)

Prepared from benzotriazol-1-ylmethyl isocyanide and dibenzylamine as solid. Method B: 12 h, 68% yield, m.p. 71-72°C Anal. calcd. for $C_{30}H_{27}N_9$ : C, 70.17; H, 5.26; N, 24.56; Found C, 70.29; H, 5.30; N, 24.69. $^{13}C$ N.m.r. δ ($CDCl_3$) 49.5, 62.4, 67.1, 108.3, 110.4, 118.9, 119.7, 123.1, 123.9, 126.3, 127.8, 132.2, 135.4, 145.3, 145.4, 155.6. $^1H$ N.m.r. δ ($CDCl_3$) 4.4 (s, 4H), 6.3 (s, 2H), 6.6 (s, 2H), 7.0-7.9 (m, 17H), 8.0-8.4 (m, 2H).

5.5.2. General Procedure for the Preparation of N',N,N-Trialkylformamidines (5.37a-f)

To a solution of N-(benzotriazol-1-yl)-N,N-dialkylformamidine (0.01 mol) in tetrahydrofuran (50 mL) Grignard reagent (0.011 mol) was added in drops. The reaction was refluxed and followed by thin layer chromatography. After the completion of the reaction, the reaction mixture was poured into a beaker containing crush ice (10 g) and hydrochloric acid (3 M, 10 mL). Extraction with diethyl ether (4 x 50 mL) removed benzotriazole. The aqueous layer is then neutralized with aqueous sodium hydroxide (20%) solution until the pH is approximately 8 (above pH 8 promoted complexation of amidine and magnesium from the Grignard reagent as yellow gel and pH below 7 gave a mixture of free amidine and protonated amidine). The

organic layer was extracted with methylene chloride (5 x 50 mL) and washed with aqueous sodium hydroxide (20%) solution (5 mL). Drying the above over anhydrous sodium sulfate and removal of the solvent under reduced pressure afforded the N,N,N'-trialkylformamidines.

5.5.2.1. Preparation of N'-benzyl-N,N-pentamethylene-formamidine (5.37a)

Prepared from N'-(benzotriazol-1-yl)methyl-N,N-pentamethyleneformamidine (5.36a) and phenylmagnesium bromide as brown oil, yield 80%. Picrate (from ethanol) m.p. 136-137°C, lit. m.p. 139°C [84HCA166]. Anal. calcd. for picrate, $C_{19}H_{21}N_5O_7$ : C, 52.90; H, 4.91; N, 16.23; Found C, 53.00; H, 4.93; N, 16.08. $^1H$ and $^{13}C$ N.m.r. data of free formamidine (5.37a) are given in Tables 5.5 and 5.6, respectively.

5.5.2.2. Preparation of N'-benzyl-N,N-(3-oxa-tetramethylene)-formamidine (5.37b)

Prepared from N'-(benzotriazole-1-yl)methyl-N,N-(3-oxa-tetramethylene)formamidine (5.36b) and phenylmagnesium bromide as yellow oil, yield 82%. Calculated for $C_{12}H_{16}N_2O$ $M^+$ 204.12625; Found $M^+$ 204.12525. $^1H$ and $^{13}C$ data are given in tables 5.5 and 5.6, respectively.

5.5.2.3. Preparation of N'-benzyl-N,N-tetramethylene-formamidine (5.37c)

Prepared from N'-(benzotriazol-1-yl)methyl-N,N-tetramethyleneformamidine (5.36c) and phenylmagnesium bromide as yellow oil, yield 79%. Calculated for $C_{12}H_{16}N_2$ $M^+$ 188.13135; $M^+$ found 188.13183. $^{13}C$ and $^1H$ N.m.r. data are given Tables 5.5 and 5.6, respectively.

5.5.2.4. Preparation of N'-(p-tolylmethyl)-N,N-pentamethyleneformamidine (5.37d)

Prepared from N'-(benzotriazol-1-yl)methyl-N,N-pentamethyleneformamidine (5.36a) and p-tolylmagnesium bromide as brown oil. Yield 62%. Calculated for $C_{12}H_{16}N_2$ $M^+$ 216.16265; M+ Found, 216.16262. $^1H$ and $^{13}C$ N.m.r. data are given in Tables 5.5 and 5.6, respectively.

5.5.2.5. Preparation of N'-(2-propenyl)-N,N-pentamethylene-formamidine (5.37e)

Prepared from N'-(benzotriazol-1-yl)methyl-N,N-pentamethyleneformamidine (5.36a) and vinylmagnesium bromide as brown oil. Yield 76%. Calcualted for $C_9H_{16}N_2$ $M^+$, 152.13135; Found 152.13012. $^1H$ and $^{13}C$ N.m.r. data are given in Tables 5.5 and 5.6, respectively.

5.5.2.6. Preparation of N'-(1-Phenyl-2-methylpropyl)-N,N-pentamethyleneformamidine (5.37f)

Prepared from N'-[1-(benzotrizol-1-yl)-2-methylpropyl]-N,N-pentamethyleneformamidine (5.36d) and phenylmagnesium bromide. During extraction precipitaed as hydrochloride salt, yield 53%, m.p. 230-232°C. $^{1}H$ and $^{13}C$ N.m.r. data are given in Tables 5.5 and 5.6, respectively.

# CHAPTER 6

## SUMMARY

The structural transformation and functional group manipulation of organic compounds using heterocyclic mediators was investigated. The two heterocyclic mediators chosen were pyrylium salts and benzotriazole. Both of these are heteroaromatic compounds possessing one ring oxygen atom and three ring nitrogen atoms, respectively.

Limitations in the existing methods of pyrylium salt application to protein characterization have been circumvented. Pyrylium salts were synthesized with various substitutions at 2,4, and 6-positions, and with various gegenions. These were reacted with lysine to transform lysine into N-(5-amino-5-carboxyl-n-pentyl)-2,4,6-tri-substituted pyridinium salts. This result of the reaction provided direct evidence of pyridinium salt formation and narrowed down the problem of stoichiometry of reaction with protein.

Two new applications of pyrylium salts in intact and isolated proteins were developed by Dill's group at Clemson University and Steven's group at the University of Florida. The two techniques involve $^{13}C$ labelling in glycophorin A,

and fluorescence labelling in cotransporter protein. Consequently, the use of pyrylium salts as specific ε-amino blocking reagents proved unequivocally that there is a head to head cross linking of the two glycophorin $A^M$ molecules via the two N-terminal L-serine residues. Reactions with increased steric bulkiness at the C-2 and C-6 positions of the pyrylium salt blocking reagent further demonstrated that there is difference in the N-terminus region of glycophorin $A^M$ and $A^N$. The most specific ε-amino blocking reagent for lysine in intact protein was found to be 4-(4-methoxy-3-sulfophenyl)-2,6-bis(4-sulfophenyl)pyrylium perchlorate.

The use of 4-(4-methoxy-3-sulfophenyl)-2,6-bis(4-sulfophenyl)pyrylium perchlorate confirmed the involvement of lysine residue(s) in the cotransporter activity of sodium ion dependent, glucose transported protein in the brush border membrane of the kidney and the intestine.

The nucleophilic ability of the benzotriazolate anion was exploited in the synthesis of both 1- and 2-isomers of hydroxyethylbenzotriazole. Hydroxyethylbenzotriazoles were further transformed into haloethylbenzotriazole derivatives. Successful nucleophilic displacement on the chloroethyl derivative has been achieved affording β-heteroatom-linked ethylbenzotriazoles.

The dual properties of benzotriazole, α-activation and leaving group ability, were found to be useful in isocyanide chemistry. Foreseeing the potential possibilities in

isocyanide chemistry, the benzotriazole group was introduced at the α-position of alkyl isocyanides.

The precursors for α-benzotriazol-1-ylalkyl isocyanides were synthesized via a facile reaction between benzotriazole, an aldehyde, and formamide to form 1:1:1 adducts, N-(benzotriazol-1-yl)alkylformamide in high yields. This preparative method for formamides is also applicable on a large scale. A general method for the synthesis of benzotriazol-1-ylalkyl isocyanides from their corresponding formamides in high yield and purity has been developed.

A novel strategy was used in the synthesis of N,N'-disubstituted and N,N,N'-trisubstituted formamides involving an electron withdrawing substituent at the α-position of an alkyl isocyanide. The combination of the electron withdrawing nature and the leaving group ability of benzotriazole at the α-position of isocyanides makes the formamidine synthesis a more versatile method. N,N,N'-trialkylformamidines with dissimilar substituents at the N and N' positions have been prepared in high yield. The leaving group ability of benzotriazole and the delocalization of electrons in the planar formamidine molecule made the method less useful for the preparation of N,N'-disubstituted formamidines.

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## Letter Codes for Journal Titles

| Code | Full Title |
|---|---|
| A | Ann. Chim. |
| AC | Acta. Cryst. |
| ACR | Acc. Chem. Res. |
| AG(E) | Angew. Chem. Int. Ed. Engl. |
| AHC | Adv. Heterocycl. Chem. |
| BBA | Biochim. Biophys. Acta |
| BCSB | Bull. Chem. Soc. Japan |
| CA | Chem. Abstr. |

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|---|---|
| CB | Chem. Ber. |
| CC | J. Chem. Soc. Chem. Commun. |
| CJC | Can. J. Chem. |
| CR | Chem. Rev. |
| HCA | Helv. Chim. Acta. |
| IC | Indian J. Chem. |
| JAM | J. Am. Chem. Soc. |
| JBC | J. Biolog. Chem. |
| JCS | J. Chem. Soc. |
| JCS(P1) | J. Chem. Soc. Perkin Trans. 1 |
| JCS(P2) | J. Chem. Soc. Perkin Trans. 2 |
| JHC | J. Heterocycl. Chem. |
| JMC | J. Med. Chem. |
| JMS | J. Mol. Spectros. |
| JOC | J. Org. Chem. |
| JP | Japan Patent |
| MI | Miscellaneous [book/journal] |
| N | Nature |
| OMR | Org. Mag. Res. |
| OSC(V) | Org. Synth. Coll. Vol. V |
| RC | Rocz. Chim. |
| RCR | Russ. Chem. Rev. |
| S | Synthesis |
| TL | Tetrahedron Lett. |
| USP | US Patent |
| USSRP | USSR Patent |
| ZOK | Zhur. Org. Khim. |

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Alan R. Katritzky, Chairman
Kenan Professor of Organic Chemistry

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August, 1989

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